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THE ORGANIC MATTER OF THE SOIL: V. A STUDY OF THE NITROGEN DISTRIBUTION IN DIFFERENT SOIL TYPES[1]

BY

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INTRODUCTION

The nature of the organic nitrogen of the soil is of more than usual interest to chemists inasmuch as this nitrogen must pass through a specific cycle of processes before it can again become generally available for the higher plants. It is perfectly obvious that these processes may be extremely rapid or very slow, depending upon the manner in which the nitrogen atoms are bound in the organic compound. For example, the nitrogen of an acid amide or an amino acid may be regarded as relatively easily converted into ammonia in contrast with such nitrogen heterocycles as pyridine or quinoline, which are probably very difficultly convertible.

It occurred to the authors that, inasmuch as soils differ widely in ammonification power, etc., it might be well to make a comparative study of the distribution of nitrogenous compounds in different soil types. Such a study was begun in the fall of 1914. Although this is the last paper of the series on the organic matter of the soil to appear, it was the first paper which was planned and to a large extent the first which was completed. Because of this fact certain of the results indicated in the preceding papers of the series could not be applied in this investigation.

[1] Received for publication February 26, 1917.

[2] The work reported in this paper was carried out in the Division of Soils, Minnesota Agricultural Experiment Station, mainly during the winter of 1914-15, the authors being respectively Assistant in Soils and Associate Professor of Soil Chemistry. These data are also a part of those recorded in a thesis presented by C. A. Morrow to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

The organic matter of the soil, containing as it does animal and plant residues, living and dead bacteria, protozoa, and fungi, and the reaction products of each upon the others, is a mixture of organic compounds of an almost hopelessly complex nature. This extreme complexity is demonstrated by the variety of organic compounds which have been isolated from soils, mainly through the work of the Bureau of Soils of the United States Department of Agriculture. Approximately 39 compounds have been isolated directly from the soil.[3] Fifteen of these contain nitrogen, i. e., cytosine, xanthine, hypoxanthine, adenine, guanine, choline, creatinine, lecithin, trimethylamine, nucleic acid, tetracarbonimide, α picoline γ carboxylic acid, arginine, histidine and lysine. The latter six of these may be considered as derived from protein materials, but the remaining nine are probably of non-protein origin. However, from other considerations, it seems probable that the protein nitrogen of the soil may exceed all other forms of nitrogen. Potter and Snyder (30) found 20.48 per cent of the alkali-soluble nitrogen of a soil to be of non-protein nature.

Historical

The chemistry of soil nitrogen may to a large extent be considered as being the chemistry of protein undergoing hydrolysis. The isolation of a number of amino acids indicates that proteins are decomposed in the soil in much the same way as in acid hydrolysis or animal digestion. Just how far the cleavages have already gone in the soil previous to acid hydrolysis remains a matter of much work before definite conclusions can be drawn.

Walters (50) has reported the presence of certain decomposition products in the soil, presumably proteoses and peptones, resulting from either a partial hydrolysis of proteins or by the synthetic action of microorganisms. They represent stages of decomposition between that of true proteins and amino acids. Walters concludes "that proteins undergo hydrolytic decomposition in the soil in much the same way as in digestion by enzymes, acids or alkalies, in the laboratory." In an extensive examination of the nitrogen compounds of processed fertilizers, Lathrop (20) has reported the presence of certain protein-like substances similar to those described above.

In his studies on the chemical nature of the organic nitrogen in the soil, Jodidi (17) thought water would be preferable to either acids or alkalies for the purpose of extraction, since it would not be so liable to alter the organic nitrogenous materials. He found that the direct extraction of a soil by boiling with water for 10 hours removed only 2.92 per cent, and for 24 hours the highest amount removed from any soil was 9.96 per cent of the total soil nitrogen. Schmook (36), however, reports 19.10 per cent of the total nitrogen of a Laterite soil of Russia to be water-soluble.

[3] This does not include several which have been isolated from soil hydrolyzed with acids, etc.

The literature has been very thoroughly summarized by Potter and Snyder (27) in regard to the determination of ammonia in soils. Both their work and that of Jodidi (16) indicate that the amount of ammonia is small. Kelley and Thompson (19) in a study of some Hawaiian soils reached the conclusion that ammonia and nitrate nitrogen constitute but a small percentage of the total nitrogen, and that the nitrogen is very largely in organic combination.

It is known that only a small part of the soil nitrogen is dissolved by dilute acids, yet it has been shown by Kelley and Thompson (19) that 1 per cent hydrochloric acid dissolves some organic nitrogen, for in every instance the soils contained only about half as much ammonia nitrogen as the total nitrogen extracted by the acid.

In the soil studies of Potter and Snyder (28) they find that the nitrogen extracted by 1 per cent hydrochloric acid varied from about 1.2 to 2.3 per cent of the total nitrogen, except in the case of the peat, where it was only 0.67 per cent. Gortner (9), working with 8 mineral soils, finds a maximum of 4.18 per cent of the total nitrogen soluble in 1 per cent hydrochloric acid with an average of 3.17 per cent. In three peats he finds a maximum of 7.50 per cent with an average of 3.78 per cent, and in 5 samples of unchanged vegetable materials (oat straw, alfalfa hay, oak leaves, sweet fern leaves, and grass from a peat bog) he finds a maximum of 34.58 per cent with an average of 20.10 per cent. These findings would seem to indicate that in the transformation of vegetable materials into the true organic matter of the soil there is a fall in the proportion of the total nitrogen soluble in very dilute acids.

Shorey (37) published results of his investigations which gave the first definite knowledge of the nitrogen distribution in the soil. Working on an Hawaiian soil he applied the method proposed by Osborne and Harris (24) for classifying the decomposition products of proteins resulting from acid hydrolysis. The method is a modification of that proposed by Hausmann (13) and is in short as follows. After hydrolysis the excess of the mineral acid is removed by evaporation, and the nitrogen present as ammonia determined by distilling with an excess of magnesium oxide. After separating the magnesia precipitate from the remaining solution by filtration, the nitrogen was determined in the precipitate by the Kjeldahl method. The di-amino nitrogen in the filtrate was precipitated by phosphotungstic acid and determined by the method of Kjeldahl and the mon-amino nitrogen determined by difference.

He obtained in the acid solution 84.5 per cent of the total nitrogen in the soil, 52.3 per cent of which was found in the magnesia precipitate. This result is in striking contrast to those obtained by Osborne and Harris (24) working on pure proteins, where they found that the nitrogen

contained in the magnesia precipitate does not usually exceed 4 per cent of the total nitrogen and in most cases is very much less. The amount of nitrogen insoluble in the 12 per cent acids used in the digestion may be designated as "humin." The nitrogen in the magnesia precipitate has been designated by most investigators as "humin" nitrogen. The total humin nitrogen in the soil is then represented by the nitrogen in the magnesia precipitate plus that retained by the soil. On recalculation of his data is was found that the insoluble humin *in the soil* after hydrolysis amounted to 15.3 per cent of the total nitrogen, making a total humin nitrogen content of 59.1 per cent. This very righ result of total humin nitrogen was undoubtedly due to the soil being hydrolyzed only 7 hours with a relatively low concentration of hydrochloric acid and the insoluble residue boiled the same length of time with sulfuric acid. Complete decomposition of the proteins probably did not take place in the dilute acids used in the short time that they were heated. As a result some proteoses and peptones were precipitated by the magnesium oxide, which would account for the high results.

Shorey (38) concluded that even though we might know much concerning the constitution of the compounds comprising the various groups isolated from protein by this method of analysis, we know nothing concerning their composition when isolated from soil, inasmuch as we are not dealing with a pure protein [*cf* also Gortner (7, 8, 10)].

The work of Suzuki (45) gives us further knowledge of the individual amino compounds formed in the decomposition of soil organic matter. He worked with three samples of humic acid, one obtained from Merck, origin unknown to Suzuki, one prepared from an unmanured soil, and one from a compost heap. After boiling each preparation for 10 hours with strong hydrochloric acid, the undecomposed residue was filtered off, washed, and the residue extracted twice in this manner with strong hydrochloric acid. He determined the amounts dissolved as amide, di-amino, and mon-amino acid nitrogen. From 65 to 75 per cent of the total nitrogen was dissolved by the hydrochloric acid and in the extract 41 to 62 per cent of the nitrogen was not precipitated by phosphotungstic acid. A sample of humic acid was twice extracted with concentrated acid and the residue analyzed. His results calculated on the ash free basis showed the residue to contain 64.11 per cent carbon, 3.35 per cent hydrogen, and 0.80 per cent nitrogen. The residue becomes lower in nitrogen[4], hydrogen, and ash but richer in carbon as the hydrolysis is continued.

Detmer (5) pointed out that similar results were true in peat beds where the deposits remain undisturbed for years. He found that there is

[4] He stated that although the nitrogen content decreases, it is very difficult to remove entirely.

an increasing carbon and nitrogen content of the humus for varying depths. This is shown by the following table:

	Carbon	Hydrogen	Oxygen	Nitrogen
Brown peat, near the surface	57.75	5.43	36.02	0.80
Dark peat, 7 feet	62.02	5.21	30.67	2.10
Black peat, 14 feet	64.07	5.01	26.87	4.05

Suzuki (43, 45) made further studies on a 500-gm. sample of the humic acid obtained from Merck. It was hydrolyzed with concentrated acid and the solution obtained subjected to esterification and fractional distillation according to the method of Fischer (6). He obtained:

Alanine	2.39 gm.
Leucine	2.16 gm.
Alanine + aminovalerianic acid	0.11 gm.
Aminovalerianic acid	0.57 gm.
Proline (copper salt of active proline)	0.67 gm.
(copper salt of inactive proline) (?)	0.50 gm.
Aspartic acid	0.06 gm.
Impure aspartic acid (?)	3.16 gm.
Glutaminic acid	present
Tyrosine	trace
Histidine	trace
Ammonia	1.90 gm.
Copper salts of unknown acids	30.30 gm.

As these compounds are typical protein decomposition products, his work proves that the humic acid examined by him was either of a protein nature, a mixture of protein decomposition products, or probably both together with some compounds as yet unknown. Unfortunately, the origin of the acid was unknown to Suzuki, but he states that it was probably prepared from peat.

From a study on Michigan peat soils, Jodidi (16) has concluded that the bulk of the organic nitrogen is made up of acid amides, di-amino acids, and mon-amino acids. He used slightly modified methods. The ammonia was determined as above by distillation with magnesium oxide. The residue from the distillation with magnesia was dissolved in dilute sulfuric acid and the di-amino acids precipitated by phosphotungstic acid. The nitrogen in the precipitate of di-amino acids was determined by the method of Kjeldahl. The filtrate from the di-amino acids containing the mon-amino acids was oxidized by the Kjeldahl method and the nitrogen determined. He secured the mon-amino nitrogen by difference in most cases, stating that it was difficult to get a direct determination of the mon-amino nitrogen by the Kjeldahl method.

He states that "this percentage was usually higher than the one directly found by Kjeldahlizing the filtrate from the phosphotungstic acid

precipitate." In one experiment the percentage of mon-amino nitrogen by direct determination was 62.83 per cent, while by difference the result was 67.22, and in another case the results were 64.25 and 65.06, respectively.

It will be noted that this is a departure from the method used by Shorey (37) in that here the nitrogen is separated into *three* fractions instead of the usual four. The nitrogen in the magnesia precipitate was distributed with the di-amino and mon-amino acid nitrogen.

Van Slyke's (47) nitrous acid method was first applied by Robinson (31) to a study of peat soil, in order to determine the amount of amino nitrogen present. The ammonia nitrogen was removed by previous distillation with magnesium oxide. The only value of Robinson's work seems to be in the fact that his figures for total and amino nitrogen increase to a maximum with increasing time of hydrolysis, in much the same manner that proteins react; thus indicating that the amino groups were not existing free in the peat but in some form of combination which did not react with nitrous acid. For example, after one hour's hydrolysis the total nitrogen of the soil in solution amounted to 29.86 per cent, while the amino nitrogen was 4.62 per cent, or a ratio exceeding 6:1. After 42 hours' hydrolysis the nitrogen of the soil in solution was 51.54 per cent of the total nitrogen and the amino nitrogen was 25.07, or a ratio only slightly exceeding 2:1. This ratio increases again with further hydrolysis so that at the end of 138 hours the ratio is almost 3:1. However, the amount of nitrogen in solution was so small that the experimental error of measuring total and amino nitrogen must have been quite large.

More recently Jodidi (17) made a study of some Iowa soils using a modification of the Osborne and Harris (24) method. The nitrogen removed from the solution by the magnesium oxide was apparently ignored by the author.[5] This contained a part of the so-called "humin" nitrogen. Subtracting the sum of the ammonia[6] and di-amino nitrogen from 100 he found the per cent of the nitrogen in solution as mon-amino nitrogen. It will be readily seen that this conclusion is incorrect. The mon-amino acid nitrogen as determined represents the sum of the humin and mon-amino nitrogen. It is very unfortunate that this mistake should have been made, since this gives us only the actual ammonia and di-amino acid nitrogen for use in comparison with other investigations of the organic soil nitrogen as distributed by acid hydrolysis. Kelley (18), following details as outlined by Jodidi (17), has made the same error and the criticisms above apply with equal force to his data.

[5] Experimental data presented later in this paper will show that this fraction may exceed 9 per cent of the total nitrogen.

[6] He distinguishes the ammonia nitrogen originally present in the soil as such, from that produced by acid hydrolysis.

It is also extremely unfortunate that investigators should in any case rely upon figures obtained "by difference" for any one of their fractions. It is sometimes permissible to use figures obtained in this manner, for example, in case a determination has been lost and lack of time or other consideration prevents a repetition, but constantly to use figures obtained in this manner is undesirable, especially, since by this method we have no means of determining how great the experimental error of the method may have been.

Jodidi (17) called attention to the fact that in the case of protein substances the distillation of the hydrolyzed protein with magnesium oxide gives pure ammonia. This, however, may not hold true for the hydrolyzed portion of soils, since some protein substances through decay yield organic bases. It has been shown by Bocklisch (3) that dimethyl amine is formed through putrefaction of fish, and trimethylamine has been produced by the putrefaction of wheat flour and fish. The bases putrescine and cadaverine result from the decay of organic substances under certain conditions. It is possible for certain di-amino acids to become transformed into di-amines, as for example, arginine can be decomposed into urea and ornithine through bacterial activity. These processes can be expressed by the following equations:

$$\underset{\text{Arginine}}{NH_2\text{-}C(NH)\text{-}NH\text{-}CH_2\text{-}(CH_2)_2\text{-}CH(NH_2)\text{-}COOH} + H_2O \rightarrow \underset{\text{Urea}}{OC\begin{matrix}NH_2\\NH_2\end{matrix}} + \underset{\text{Ornithine}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH}$$

$$\underset{\text{Ornithine}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH} \rightarrow CO_2 + \underset{\text{Putrescine}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2}$$

Jodidi found that the ammonia obtained by distilling the evaporated extract of the soil with magnesium oxide, was actually pure ammonia, thereby establishing the absence of any volatile organic bases; but concluded that the phosphotungstic acid precipitate and the filtrate from that precipitate did not represent di-amino and mon-amino acids only.

In order to find out how much of the di-amino and mon-amino nitrogen actually belongs to diamino and mon-amino acids, the solutions were subjected to analysis by the formaldehyde-titration method of Schiff (33, 34, 35) as modified by Sörensen (41), Henriques (14) and Henriques and Sörensen (15).

In a comparison of the amount of di-amino[7] acid nitrogen, calculated as if histidine, arginine, and lysine were present in about equal amounts, he finds that in Plot E, 101.8 per cent; in Plot Q, 84.8 per cent; and in Plot U, 93.9 per cent of the nitrogen in the phosphotungstate fraction was actually present as di-amino acids.

[7] The exact interpretation of his data is difficult to understand.

However, he obtains widely divergent results for mon-amino acid nitrogen, in Plot E, 91.64 per cent; in Plot Q, 52.63 per cent; in Plot U, 40.12 per cent; in Plot H, 88.31 per cent; and in Plot J, 92.11 per cent of the total nitrogen in the "filtrate from the bases" was actually present as mon-amino acid nitrogen, whereas all should have been present in this form if he were dealing with pure proteins only. He, therefore, concludes that the di-amino and mon-amino acids, or in other words *the bases and filtrate from the bases obtained by hydrolyzing soils, contain other products than are formed by hydrolysis of pure protein.*

In a series of fertilized soils studied by Lathrop and Brown (22) they find that almost 98 per cent of the nitrogen in the soil is of organic nature. The ammonia and the nitrate nitrogen constitute the remainder. Employing the same method for the distribution of the soil organic nitrogen as Shorey (37), they boiled 100 gm. of soil with 500 c.c. of hydrochloric acid (sp. gr. 1.115) for 3 hours, and used the filtrate after making to definite volume for the analyses. The figures given for ammonia nitrogen represent the actual amount of nitrogen as ammonia obtained by hydrolysis and do not include the ammonia nitrogen already present in the soil. They find that the plots which have received organic fertilizers give the largest amount of ammonia on hydrolysis, the amount being highest in the plot which has received manure alone and lowest in the check plot.

Of the five soils studied, four contained over 26 per cent of the "humin" nitrogen soluble in acid, while the other showed only about half as much. Since the nitrogen of the soil not soluble in acid may be considered "humin" nitrogen, the total amount in this form in the above four soils was over 53 per cent, while in the other soil, which received dried blood, it amounted to only 43 per cent.

However, the fractions which they determined have actually very little significance in a discussion of protein hydrolysis products, inasmuch as a 3-hour hydrolysis is far too short a time to decompose the protein molecule completely. This explains their high figures for humin nitrogen and low ones for mon-amino acid nitrogen. The figures for di-amino and mon-amino acid nitrogen differ rather widely, but there seems to be no agreement between the form of nitrogen and the plot treatment.

In conclusion they say "these five samples of soil are really the same soil under long continued treatment of different kinds. It is not improbable that work on widely different soils will show even much greater variations than those here noted. The work shows, however, that even in such cases there is a difference in the nitrogenous compounds in the soil, and that different decompositions of the nitrogenous matter have taken place and probably will continue to take place, under the different conditions imposed upon the soils in the field."

A very interesting study has been made by Shmook (36) of the nitrogen distribution in four Russian soils, one of the Podzol type, two of the Chernozem type, and one of the Laterite type, by applying the method of Hausmann (13). The water extract from 100 gm. of the Podzol soil showed a very high content of soluble nitrogenous compounds. This amounted to 0.0452 gm. of nitrogen which constituted 19.10 per cent of the total soil nitrogen. This was distributed as follows: amide nitrogen, 0.0034 gm.; di-amino nitrogen, traces; and mon-amino nitrogen, 0.0408 gm. These results were deducted from the analyses of the hydrolyzed soil.

He finds that the Chernozem and Podzol soils show a similarity in the distribution of amide nitrogen, and that of the amino acid nitrogen, but that the nitrogen distribution in the Laterite soil is entirely different from that of the other types. He concludes that the amount of protein in the soil is not in direct relation to the amount of organic matter, and that the nitrogen insoluble in hydrochloric acid occurs in unknown form and composes only 1.50 to 1.90 per cent of the organic matter of the Chernozem and Podzol soils, but 13.70 per cent of the total organic matter of the Laterite soil after subtraction of the protein nitrogen belonging to this insoluble portion. He suggests these results would indicate that the organic nitrogen existed in the soil in large part as protein material in the Chernozem and Podzol soils, but that a considerable portion was of a non-protein origin in the Laterite soil, since the amount of this insoluble "melanin" in pure proteins amounts to from 0.60 to 1.80 per cent of the total protein nitrogen.[8]

Potter and Snyder (28) made a study of some Iowa soils, using Van Slyke's (47) method of protein analysis. Their soils were of the same type but had received different fertilizer treatment. At the same time they also made a study of a peat soil. The soils were in all cases first extracted with 1 per cent hydrochloric acid "in order to render the humus more soluble." They were then hydrolized by boiling one part of the soil with two parts of 22 per cent hydrochloric acid for 48 hours. They also prepared a 1 per cent sodium hydroxide extract of the acid-leached soils and after precipitation with sulfuric and acetic acids the resulting "humic acid" precipitate was subjected to the above method of analysis.

The authors conclude: (a) that the humin nitrogen as determined by the Van Slyke method in the dilute alkali extract of soils is very high when compared with the amounts in proteins; (b) that no typical class of organic compounds is extracted from the soil by dilute alkali; (c) that

[8] Actually in some cases these results are much lower, and in others are decidedly higher, e. g., Van Slyke (46) finds gelatin contains 0.07 per cent and fibrin 3.17 per cent.

the amounts of amino acid and peptide nitrogen in the soil are found to be very small compared with the amounts of amino acids formed by acid hydrolysis; (d) (and this is the most important for our purpose) that "nothing very significant can be deduced from the variations in the different soils," or in other words, *the organic nitrogen in the same soil type under different fertilizer treatment is essentially the same, and as we shall show later in the experimental part of this paper, the organic nitrogen (as distributed by Van Slyke's method) in different soil types is essentially the same.*

Lathrop (21) recently made a study of protein decomposition in the soil. He added a high-grade nitrogenous fertilizer to the soil and allowed decomposition to proceed at laboratory temperature, and at different periods took samples and subjected them to Van Slyke's method of protein analysis in order to determine how the different fractions were affected by bacteria and other agencies present in the soil.

From his work he concludes that the analysis obtained by the Van Slyke method indicates that there is a formation of protein taking place in the soil in the course of the decomposition of the protein materials, and that apparently the new protein is somewhat resistant to decomposition. He states that "this is indicated in (a) the unequal loss of monamino acids and hydrolyzable nitrogen from the soil during the early stages, (b) by an increase in amide nitrogen during the early stages, (c) by an increase in histidine nitrogen during the early stages, (d) by an increase in the arginine nitrogen during the later stages, and (e) by an increase in lysine nitrogen during the later stages." This view that the protein nitrogen in the soil was largely contained in the bodies of bacteria and protozoa had been previously advanced by Shmook (36).

It was stated by Loew and Aso (23) that under favorable conditions of growth protein material is excreted by yeast and bacteria, and that soluble materials can pass through the cytoplasm to the outside on the death of the cell. They also state that the amount of nitrogenous substances partly consisting of peptones excreted by dead cells, is by no means inconsiderable.

Experimental

The problem. It has been shown in the historical study above that a number of investigators have studied the distribution of the organic nitrogen in the soil by applying either Hausmann's (13) or Van Slyke's (47) method of protein analysis. It has been demonstrated by Potter and Snyder (28) that various plots on a single soil type but under different cultural conditions gave, with Van Slyke's method, essentially the same nitrogen distribution.

The results of Potter and Snyder's work were published some time after the present investigation had been begun, but the problems in the

two instances were not exactly similar. We have made a study of the distribution of the organic nitrogen in *different soil types* in an attempt to see whether the forms in which nitrogen occurs differ from locality to locality, and from soil type to soil type.

The material. This study was made using two peats, one muck, seven mineral surface soils and one mineral subsoil. All but one of the soils used are from samples of soils which have been used in the preceding papers of this series. Inasmuch as a complete description of these soils has already been given (9, 11), it will not be repeated.

The soils used were Fargo silt loam, Fargo clay loam, forest-covered loess, prairie-covered loess, Hempstead silt loam, Carrington silt loam (2 samples),[9] Hempstead silt loam subsoil, sphagnum-covered peat, black peat and "muck."

All of the samples were used in an air-dry condition.

The method. The method of Van Slyke (47, 48) has been used throughout this investigation because the nitrogen can be separated into a larger number of fractions than when the earlier method of Hausmann (13) is employed. The different fractions, however, are not listed in the same manner as in the Van Slyke method, for since we are not dealing with pure protein material we cannot correctly speak of arginine, histidine, cystine, and lysine nitrogen.

Van Slyke (49) has called attention to the fact that his method was devised for the analysis of *pure protein* material and not for a heterogeneous mixture of nitrogen compounds. This fact is apparently not recognized by certain investigators.

It is obvious that there are other types of organic materials which will interfere with the nitrogen distribution. It seems very probable that in plant materials and in soils there must be many organic nitrogenous compounds which have no relation to the protein molecule, such as purine bases, pyrimidine bases, nitrogenous fats, nitrogenous pigments, as well as other non-protein nitrogenous compounds. Much valuable *comparative* data can be obtained by the application of Van Slyke's method to the analysis of heterogeneous materials; but it is self-evident that no analogy can be drawn between the analysis of pure protein and the analysis of a protein mixed with an unknown amount of foreign nitrogenous compounds. The results obtained from the hydrolysates of soils are of little value in advancing our knowledge of soil proteins or for comparison with analyses of pure proteins, but may be extremely valuable and

[9] One sample of Carrington silt loam was from Nerstrand, Rice County, Minnesota, situated on the Kansas glacial drift. This sample was used in the earlier papers of this series. The other sample was from Morristown, Rice County, Minnesota, situated on the Late Wisconsin glacial drift, and had not been used in the preceding studies. Like the sample from Nerstrand, it represents a composite of 100 borings to a depth of 6 inches, 20 borings being taken from each of 5 virgin fields.

interesting for comparison between themselves and with other analyses of soils carried out under similar conditions. It must be remembered that all data on similar material are strictly comparable when the same method of analysis is followed.

It is possible that many of the non-protein nitrogenous compounds may be split up during the hydrolysis of heterogeneous material. Gortner (7) has shown that uric acid nitrogen is distributed in all four of the major fractions after hydrolysis. The ammonia nitrogen amounted to 15.27 per cent, humin nitrogen 35.98 per cent, basic nitrogen 12.97 per cent, and non-basic nitrogen 35.78 per cent. "The humin nitrogen contained no trace of black color and was probably calcium ureate." Probably all of the purines and pyramidines would behave in a similar manner.

The general method employed in this investigation will be discussed in detail for two soils, a peat and a mineral soil, inasmuch as the experimental conditions vary in minor details with the two types.

1. *The method in detail for a peat soil.* Duplicate samples were hydrolyzed in the presence of hydrochloric acid for 48 hours. The content of calcium oxide was taken into account, and corrections made so that the hydrochloric acid used was of sufficient concentration to neutralize the lime and at the same time furnish a constant boiling acid. The hydrolysis was carried out in 200-c.c., long-neck, round-bottom Kjaldahl flasks, fitted with Hopkins' condensers made from a test tube which fitted rather loosely into the neck of the flask. The flasks were heated to gentle boiling on the same sand bath over an Argand burner, so that the rate of hydrolysis would be as nearly the same as possible.

After completion of the 48-hour hydrolysis the mixture was evaporated in a Claissen distilling flask under diminished pressure until all the hydrochloric acid possible was driven off. The residue after this distillation was dissolved in 100 to 150 c.c. of water, 100 c.c. of 95 per cent alcohol, and an excess of calcium hydroxide suspended in water was added and the ammonia distilled off into standard acid at a temperature of 40-50° under a pressure of less than 30 mm., distillation being continued for at least a half-hour. The results are listed under "ammonia nitrogen."

The alkaline mixture in the distilling flask was filtered and the precipitate well washed with hot water until free of chlorides. A Kjeldahl determination was made of the filter and its contents, and the results listed under "humin nitrogen."

The filtrate and washings from the humin were acidified with hydrochloric acid and concentrated under diminished pressure to less than 200 c.c., and to this solution was added 18 c.c. of concentrated hydrochloric acid and the whole heated on the water bath until hot. A solution containing 15 gm. of phosphotungstic acid was then added and the

heating on the water bath continued for an hour. The flask was then set aside in a cool place for 48 hours. The precipitate of the bases was then filtered off and washed as directed by Van Slyke (47).

The basic phosphotungstates were suspended in 800 c.c. of water and brought into solution by the cautious addition of a 50 per cent solution of sodium hydroxide, a few drops of phenolphthalein being added to guard against too great an excess of alkali. The phosphotungstic acid was precipitated by the addition of a slight excess of 20 per cent barium chloride, and the barium phosphotungstate was filtered off and washed free of chlorides with hot water.

The filtrate and washings were united, acidified with hydrochloric acid, and concentrated under diminished pressure to a very small volume. After cooling, any residue was filtered off, washed, and the filtrate made to 50 c.c. volume.

The washed precipitate of barium phosphotungstate and filter were subjected to Kjeldahl determination for any nitrogen that might be held by absorption, adsorption, or occlusion, as was also any residue remaining on the filter after the final filtration of the solution containing the basic nitrogen. In all cases some nitrogen was found. This nitrogen is probably derived from the "unadsorbed humin carried down with the basic phosphotungstates" mentioned by Van Slyke (49, p. 284). Inasmuch as this work was done prior to Van Slyke's publication, we added this nitrogen to the total nitrogen content of the bases instead of to the humin.

In no case did we attempt to separate the basic nitrogen into the usual fractions of "arginine," "cystine," "histidine," and "lysine" nitrogen, because we were not dealing with pure protein material. The nitrogen of the arginine determination is listed as "nitrogen set free as ammonia by 50 per cent potassium hydroxide." The solution remaining from this determination was used in the estimation of the total nitrogen of the bases. This was performed according to Van Slyke's directions. The quantity of acid neutralized in this determination was added to that neutralized in the nitrogen set free as ammonia by 50 per cent potassium hydroxide, thus securing the "total basic nitrogen."

The "amino nitrogen of the bases" was determined in Van Slyke's (48) apparatus, using 10-c.c. portions of the solution.

The filtrate from the bases was treated with sodium hydroxide solution until a slight turbid precipitate of lime was formed, and then cleared immediately by the addition of acetic acid. The solution was then concentrated under diminished pressure and on cooling was made to 200 c.c. volume. "Total nitrogen in the filtrate from the bases" was determined on duplicate portions of 25 c.c. each by the method of Kjeldahl. The digestion was continued for 3 hours after the solutions were clear, so

that the phosphotungstic acid would not interfere with the accuracy of the determination. The "amino nitrogen in the filtrate" from the bases was determined on duplicate portions of 10 c.c.

2. *The method in detail for a mineral soil.* Duplicate portions of 250 gm. were hydrolyzed in round-bottom Kjeldahl flasks for 48 hours on different sand baths. Allowance was always made for the lime content of the soil, and the requisite amount of hydrochloric acid added to insure the presence of a constant boiling acid (sp. gr. 1.115), and a volume of approximately 250 c.c. The solutions boiled smoothly and gave no trouble by bumping.

On completion of the hydrolysis the two samples were diluted to 1000 c.c. in measuring flasks and allowed to settle for at least 24 hours. The clear solution was then siphoned off and an aliquot of 500 c.c. analyzed according to the usual method of Van Slyke. In nearly all cases this solution was straw color, as a result of the presence of ferric salts that had been formed during the hydrolysis. No black color, the usual color of a protein hydrolysate, was observed in any instance.

The soil remaining in the measuring flask was washed free from soluble nitrogen with a 1 per cent solution of potassium sulfate, by decantation from tall soil beakers, the solution after settling being siphoned off not oftener than twice a day. The electrolyte was added in order to prevent the clay from forming a colloidal suspension, and at the same time the particular salt chosen would not interfere with the subsequent Kjeldahl determination.

A concrete example of the thoroughness of this washing may well be given. It will be noted that 700 c.c. of the original hydrolysate was siphoned off for the different analyses. This left a total volume of 300 c.c. of residue and solution to be washed by decantation with 1 per cent potassium sulfate. By the methods of calculation given in the following paragraphs it was found that the remaining solution contained 0.1089 gm. of nitrogen. If three-fourths of the wash solution is removed each time, there will remain in the solution at the end of the fourth washing approximately 0.0004 gm. of the original nitrogen. Actual Kjeldahl determinations were made on 250-c.c. portions from the fourth washing in the case of duplicates from the same soil, and the results indicated that 0.0006 gm. of nitrogen still remained in the solution. Since this was within experimental error of the theoretical value, the method of washing by decantation was followed in all the subsequent work with mineral soils, or those which had mineral soils added previous to the analysis.

The residue from the hydrolyzed soil was evaporated to dryness on the steam bath in an evaporating dish, then further dried at about 110° C. After cooling, this dry soil was passed through a 1-mm. sieve and after being thoroughly sampled, duplicate nitrogen determinations were made

on 15-gm. portions and the total nitrogen remaining in the soil calculated. These results are listed as "insoluble humin nitrogen in the soil." The weight of the dry soil divided by the average specific gravity (2.6) represented the actual volume occupied by this soil residue. The total volume of the hydrolysate minus the volume occupied by the insoluble residue gives the actual volume of the soil solution.

Since the analysis was made on 500 c.c. of the soil solution it was necessary to recalculate the total "insoluble humin nitrogen in the soil" in order to determine the amount of this humin nitrogen actually belonging to the aliquot analyzed.

The total nitrogen belonging to the solution analyzed was found by taking the sum of the total nitrogen in the solution and the above insoluble humin nitrogen. Knowing the total nitrogen content of the soil before hydrolysis and the total nitrogen in solution, the per cent of the total nitrogen in solution after hydrolysis can be determined.

The 500-c.c. aliquot was concentrated under reduced pressure until the greater part of the hydrochloric acid had been removed and the ammonia nitrogen determined in the manner outlined under the peat analysis.

The "humin" fraction precipitated by the calcium hydroxide was almost colorless or light yellow because of the iron salts contained in it. This bulky precipitate was always washed by decantation after the method above described, except that distilled water was used, the united washings being concentrated to 200 c.c. or less for the precipitation of the basic nitrogen.

It was found necessary to use 35 gm. of phosphotungstic acid for the precipitation of the bases. The remainder of the analysis was carried out as directed under peats.

3. *The determination of nitrogen.* Nitrogen was determined on the soils and soil extracts in the usual manner, 25 to 35 c.c. H_2SO_4, 10 gm. K_2SO_4, and a crystal of $CuSO_4$ being used. All titrations were made with N/14 acid and alkali so that the figures obtained represented milligrams of nitrogen without necessitating a calculation.

The Analytical Data

1. *Analysis of "fibrin from blood" hydrolyzed in the presence of* 100 *gm. of ignited subsoil.* This analysis was conducted in order to ascertain, if possible, the effect of soil minerals upon the hydrolysis of a pure protein. Fibrin was selected because it was from a sample already analyzed (10). The subsoil was first ignited to redness in a muffle furnace for an hour, in order to drive off all the organic matter, and subsequently cooled in a desiccator.

Duplicate portions of 5 gm. of fibrin and 100 gm. of ignited subsoil were hydrolyzed in the presence of hydrochloric acid. The analysis was

conducted as described for the mineral soils, excepting that a 600-c.c. aliquot was used for the analysis, this amount of solution being equivalent to 3 gm. of fibrin.

The experimental data showing the grams of nitrogen found and per cent of total nitrogen are given in Table I.

Table II shows a comparison of these analyses with other analyses of the same sample of fibrin hydrolyzed alone, and in the presence of three times its weight of cellulose [data of Gortner (10)].

Differences between these analyses, together with differences between duplicates in each series of analysis, and data showing "maximum" and "average" exprimental differences to be expected are given in Table III.

These comparisons will be considered in detail under "Discussion" in the latter part of this paper.

2. *Sphagnum-covered peat.* Duplicate samples were hydrolyzed in the usual manner. It was found in the ammonia determination that all of the ammonia nitrogen could not be driven off in a half-hour when the volume of solution was large and a bulky precipitate of iron and aluminum hydroxides was present. Continued distillation for a further half-hour in this case gave additional ammonia nitrogen amounting to 0.0060 gm. In all subsequent work with both peats and soils the volume was kept smaller by the use of a more concentrated suspension of calcium hydroxide and the distillation was usually continued for one hour.

The calcium hydroxide precipitate containing the "humin" nitrogen was difficult to digest in the subsequent Kjeldahl determination, because of the large amount of organic material present. From 100 to 200 c.c. of sulfuric acid was required for the digestion. After digestion the material was transferred to a 1000-c.c. flask and 250-c.c. portions used for the distillation.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

3. *Sphagnum-covered peat hydrolyzed in the presence of nine times its weight of mineral subsoil.*[10] Duplicate 10-gm. samples were hydrolyzed in the presence of 90 gm. of subsoil with constant boiling hydrochloric acid for 48 hours.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

A comparison between these analyses and those of the peat hydrolyzed alone is made in Table IV, the data of the peat hydrolyzed alone being recalculated from a 15-gm. basis to a 10-gm. basis.

4. *Sphagnum-covered peat hydrolyzed in the presence of metallic tin.* The peat was hydrolyzed in the presence of a reducing agent because

[10] This was the first attempt to determine the nitrogen fractions in the presence of a mineral soil. For certain reasons later analyses have already been reported in this paper. The analyses as reported in this paper are by no means in chronological order, a fact which may explain seeming inconsistencies.

it was thought that possibly the amount of "humin" nitrogen would be reduced, for according to Samuley (32) the formation of this dark-colored product is due to an oxidative process [*cf.* Hlasiwetz and Habermann, and Cohn, cited by Plimmer (26, p. 17)].

It is perhaps significant that the "humin" nitrogen was reduced 3.88 per cent by the presence of a reducing solution. It is not known whether there was sufficient tin present to maintain a reducing solution throughout the hydrolysis, inasmuch as the ferric iron in the peat would have an oxidizing action on the stannous salt. The sample was known to contain iron but the amount was not determined.

Duplicate 15-gm. samples were hydrolyzed with 100 c.c. of hydrochloric acid (sp. gr. 1.115) for 48 hours in the presence of 5 and 10 gm. of tin, respectively. The tin was first partially dissolved in the acid before the samples of peat were added.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

5. *Calcareous black peat.* Duplicate samples of 15 gm. were hydrolyzed for 48 hours in the presence of 100 c.c. of constant boiling hydrochloric acid, and the analysis conducted as described under the method for a peat soil.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

6. *Acid "muck" soil.* One 25-gm. sample was hydrolyzed in the presence of 125 c.c. of concentrated hydrochloric acid for 48 hours.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

7. *Fargo clay loam.* Duplicate portions of 250 gm. were hydrolyzed for 48 hours.

The "humin" precipitate required 100 c.c. of sulfuric acid for the digestion. After digestion the material was transferred to a 500-c.c. flask and 250-c.c. portions used for the distillation. This method was followed subsequently with the "humin" nitrogen determination of all the mineral soils.

The addition of 15 gm. of phosphotungstic acid to the filtrate from "humin" did not entirely precipitate the bases. Five-gm. portions were added from time to time until a total of 50 gm. had been used. After standing the usual length of time the precipitate of the bases was filtered off, but even then the wash water caused the formation of a small additional precipitate in the filtrate. After warming on the steam bath this final solution was perfectly clear, and on standing over night, only a trace of precipitate was formed; so the precipitation was considered complete. It appears probable that a portion of this precipitate is due to the formation of inorganic phosphotungstates which consume a very large

amount of the phosphotungstic acid, for if all of this precipitate had consisted of basic nitrogen compounds the amount of nitrogen recovered should have been greater than the amount which was actually found. In all the subsequent work 35 gm. of phosphotungstic acid was used for the precipitation of the bases in the hydrolysates from mineral soils. The phosphotungstate precipitate dissolved very slowly in the sodium hydroxide, as did all other phosphotungstic acid precipitates of the mineral soils studied.

During the concentration of the filtrate from the bases so much precipitate separated, that this was filtered off and the solution made up to 300 c.c. volume. The salt remaining was dissolved in water and also made up to a volume of 300 c.c. Aliquot portions were taken from each solution and combined for the different determinations.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

8. *Fargo silt loam.* Two 125-gm. portions were hydrolyzed for 48 hours with 500 c.c. of hydrochloric acid (sp. gr. 1.18).

The resulting hydrolysates from the two flasks were combined and diluted to 2 liters. After settling, a 1-liter portion was siphoned off and analyzed by the usual method. Two 100-c.c. portions were used for the determination of total nitrogen in solution.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

9. *Carrington silt loam.* Sample I represents soil from Nerstrand, Minnesota, situated on the Kansan drift, and Sample II from Morristown, Minnesota, situated on the Late Wisconsin. A single hydrolysis of 250 gm. was made in each instance.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

10. *Hempstead silt loam.* Duplicate 250-gm. samples were hydrolyzed for 48 hours.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

11. *Prairie-covered loess.* In Sample I, 250 gm. were hydrolyzed in the presence of 250 c.c. of constant boiling hydrochloric acid for 48 hours. The hydrolysate on cooling was diluted to 1000 c.c. and a 500-c.c. portion was siphoned off and used for the analysis. In Sample II, two 125-gm. samples were hydrolyzed in the same manner outlined under Fargo silt loam (500 c.c. of constant boiling hydrochloric acid to 125 gm. of soil). The dilution and aliquot used for analysis were also the same.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

12. *Forest-covered loess.* In Sample I, 300 gm. of soil were hydrolyzed and diluted in the same manner as Sample I of prairie-covered loess. In Sample II, 300 gm. of soil were hydrolyzed under the same conditions as Sample II of prairie-covered loess.

The volume of acid used in the hydrolysis had but little effect on the proportion of the different fractions. The only observed difference was in the insoluble humin nitrogen retained by the soil residue, and this was slightly larger in Sample II, which was hydrolyzed in the presence of the greatest excess of acid. In connection with this it must also be noted that there was a somewhat larger quantity of nitrogen in solution in Sample II than in Sample I. Much the same results were found with the prairie-covered loess. All increases or decreases in the various fractions due to the greater excess of acid may well be considered to be within the experimental error.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

13. *Analysis of a 1 per cent hydrochloric acid extract of sphagnum-covered peat.* Acid extraction was made of the two peats in direct contact with the 1 per cent hydrochloric acid. For the extraction 125-gm. portions were placed in 2.5-liter acid bottles and 2 liters of 1 per cent acid added, a total of 500 gm. of peat being used. The bottles were shaken at intervals during 5 days and then the contents filtered through two thicknesses of cheese-cloth and squeezed in the hands. The resulting solution was then filtered through two thicknesses of filter paper on a Büchner funnel. This extract was light-straw colored.

It has been shown by a number of investigators, *e. g.*, Jodidi (16), Kelley and Thompson (19), and Gortner (9), that considerable amounts of nitrogen are dissolved from certain soils by this preliminary treatment. The acid solution thus obtained should contain the ammonia, acid amides, amines, amino acids, and all other organic nitrogenous compounds soluble in water or very dilute acid. The 1 per cent hydrochloric acid extracted 8.57 per cent of the total nitrogen from the peat.

Duplicate nitrogen determinations were made on 250-c.c. portions of the acid extract and from these results the total nitrogen in the bulk solution determined. The 5500 c.c. of solution containing 0.6468 gm. of nitrogen were used for analysis. This solution was concentrated under reduced pressure to about 200 c.c. and then hydrolyzed for 48 hours, after 75 c.c. of concentrated hydrochloric acid were first added. During evaporation under reduced pressure considerable hydrolysis took place, for the solution turned dark brown in color.

The analysis of this extract from the sphagnum-covered peat shows that over 65 per cent of the nitrogen is in the form of ammonia. Potter and Snyder (28) have shown that a very small amount of the nitrogen

in the 1 per cent hydrochloric acid extract of soils exists in the soil as ammonia nitrogen. It seemed probable that if an extract of the peat contained so much ammonia nitrogen after hydrolysis, the air-dry peat must contain an appreciable amount in the ordinary condition. The ammonia nitrogen was determined directly on a 5-gm. sample of the air-dry material. An excess of calcium hydroxide solution was added and the mixture distilled under reduced pressure for 45 minutes. It was found that 5.40 per cent of the total nitrogen of the soil existed in the form of ammonia nitrogen.

The precipitate containing the "humin" nitrogen was washed by decantation until practically all the dissolved nitrogen was removed. After digestion the material was diluted to 500 c.c. and 250-c.c. portions used for distillation. Before concentration the filtrate from the "humin" precipitate was a reddish color, and when finally concentrated a cherry red.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

14. *Analysis of that portion of sphagnum-covered peat soluble in 4 per cent sodium hydroxide and precipitated by hydrochloric acid.* The organic material soluble in 4 per cent sodium hydroxide was next extracted from new portions of the peat. Twelve 5-gm. portions were leached with 1 per cent of hydrochloric acid to the absence of calcium and the excess of acid removed by washing with distilled water, until the filtrate indicated only a faint trace of free acid when tested with Squibb's litmus paper. After leaching and washing, each 5-gm. portion was washed into tall glass-stoppered cylinders of 500 c.c. capacity with 4 per cent sodium hydroxide, and filled to the mark. These were thoroughly shaken and placed on their sides, thus allowing the peat to settle on the sides of the cylinder and exposing a very large surface to the action of the hydroxide. The shaking was repeated at intervals for 9 days. The cylinders were then thoroughly shaken, placed in a vertical position and allowed to settle for 4 days before the supernatant liquid was siphoned off and filtered.

These filtered solutions were neutralized with hydrochloric acid (solution tested faintly acid) when a brown flocculent precipitate separated. This was allowed to settle for several hours and the cider-colored solution siphoned off. The brownish-black precipitates were filtered and after draining over night were thoroughly mixed with a large volume of water and again filtered and drained. The resulting precipitates were hydrolyzed with 200 c.c. of hydrochloric acid for 48 hours. This amount of concentrated acid was added and the flask brought to boiling until hydrogen chloride fumes were evolved, showing the presence of constant boiling acid.

The entire hydrolysate was used for the ammonia determination. After this determination the "humin" precipitate was thoroughly ground in a mortar to insure complete disintegration, although this seemed hardly necessary, as the solid was already in a fairly fine state of division. This precipitate was washed in the usual manner by decantation, the filtrate concentrated by evaporation and made to 250 c.c. volume. Duplicate portions of 25 c.c. were used for the determination of total nitrogen in the solution. The remaining 200-c.c. portion was used for precipitation of the bases and subsequent analysis.

The high content of carbonaceous organic matter made the "humin" precipitate very difficult to digest. The sulfuric acid required was 130 c.c. and the digestion extended over 10 days before complete decoloration was effected. Of course, precautions were taken to prevent the absorption of ammonia from outside sources. The material was diluted to 500 c.c. and 250-c.c. portions used in the distillation.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

15. *Analysis of that portion of sphagnum-covered peat soluble in* 4 *per cent sodium hydroxide and not precipitated by hydrochloric acid.* The filtrates remaining from the brownish-black precipitate formed by acidifying the sodium hydroxide extracts of the soil with hydrochloric acid (*cf.* Section 14) were concentrated in the usual manner to about 700 c.c., when a heavy precipitate of sodium chloride separated. On standing over night there also separated a heavy flocculent brown precipitate. This may have been due to the salting out effect of the sodium chloride on some of the organic substances in the solution. The solution was saturated with hydrogen chloride in the cold and the mixture then divided into two portions and hydrolyzed for 48 hours. After hydrolysis the portions were united and filtered through glass wool and the precipitate washed with concentrated hydrochloric acid. The filtrate was allowed to stand in a tall soil beaker when more salt separated. This was due to the increased concentration of the hydrochloric acid. The salt that separated was freed from the mother liquid by packing in a centrifuge and washing a number of times with acid. The salt, washed as free of the solution as possible, was dried on the steam bath. It was nearly white in color. The glass wool was dried and ground with the salt. After being sampled, 15-gm. portions were used for Kjeldahl determinations. The results were listed as "nitrogen retained by the salt."

The combined filtrates were concentrated and analyzed.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

16. *Summary Tables.* Certain of the preceding analyses have been summarized in Tables V and VI.

In Table V are shown the amounts and percentages of soil dissolved by the acid during hydrolysis as well as the amount of nitrogen and percentage of the total nitrogen dissolved.

Table VI summarizes the average nitrogen distribution of all the soils analyzed. Because of the large amount of space required, the tables showing the analyses in detail, both as regards grams of nitrogen in the different fractions and the concordance of duplicate determinations, have been omitted, and only the summary table is given. The detailed tables will ultimately be available in a printed thesis from the Graduate School of the University of Minnesota.

TABLE I

NITROGEN DISTRIBUTION IN THREE GRAMS OF MERCK'S "FIBRIN FROM BLOOD" HYDROLYZED IN THE PRESENCE OF 100 GRAMS OF IGNITED SUBSOIL

	Gm. Nitrogen		Per Cent Total Nitrogen		
	I	II	I	II	Av.
Total N	0.4577	0.4591	.....	.....	.....
Ammonia N	0.0457	0.0455	9.98	9.91	9.95
Insoluble humin N in soil	0.0125	0.0130	2.73	2.83	2.78
N precipitated by $Ca(OH)_2$	0.0220	0.0221	4.81	4.81	4.81
Basic N[1]	0.1219	0.0995	26.63	21.68	24.15
Arginine N	0.0598	0.0440	13.07	9.58	11.32
Histidine N	None	None	.....	.....	None
Lysine N	0.0597	0.0531	13.04	11.57	12.30
Cystine N	.....	.....	.....	.....	[2]0.51
Amino N in bases	0.0775	0.0746	16.93	16.25	16.59
N in filtrate from bases	0.2725	0.2909	59.54	63.36	61.45
Amino N in filtrate from bases	0.2671	0.2702	58.36	58.85	58.61
Non-Amino N in filtrate from bases	0.0054	0.0207	1.18	4.51	2.84
Total N regained	0.4746	0.4710	103.69	102.59	103.14

[1] The barium phosphotungstate retained 0.0022 gm. of nitrogen in Sample I and 0.0020 gm. in Sample II.

[2] From data of Gortner (10).

Discussion

Changes in nitrogen distribution in a protein when hydrolyzed in the presence of a mineral soil. From a study of Table II it is seen that the histidine nitrogen of fibrin hydrolyzed alone is 4.36 per cent and when hydrolyzed in the presence of cellulose it is 4.86 per cent of the total nitrogen. However, when fibrin is hydrolyzed in the presence of ignited subsoil we see the histidine nitrogen is entirely lacking[11] and that the nitrogen precipitated by calcium hydroxide amounts to 4.81 per cent. This corresponds very closely to the amount of histidine found in the other two cases. In other points the three analyses agree within experimental error.

[11] It is of interest to note that Brewster (4) found a similar behavior in the hydrolysis of vegetable materials. On hydrolyzing whole Kafir grain he obtained no histidine fraction, while the pure protein of Kafir grain showed a histidine content of 1.64 per cent.

It appeared possible that the histidine nitrogen might have been converted into the nitrogen fraction precipitated by calcium hydroxide. It is well known that histidine can be precipitated by silver salts in slightly alkaline solutions; and it was thought possible that the histidine might be precipitated by some of the mineral constituents of the soil and thus be found with the calcium hydroxide precipitate.

TABLE II

COMPARATIVE ANALYSES OF THREE GRAMS[1] OF MERCK'S "FIBRIN FROM BLOOD" HYDROLYZED ALONE AND IN THE PRESENCE OF CARBOHYDRATE AND OF IGNITED SUBSOIL

	3 gm. of Fibrin hydrolyzed alone [data of Gortner (10)]	3 gm. of Fibrin hydrolyzed in the presence of 9 gm. of cellulose [data of Gortner (10)]	3 gm. of Fibrin hydrolyzed in the presence of 100 gm. of ignited subsoil (average data of Table I)
	Per cent total nitrogen	Per cent total nitrogen	Per cent total nitrogen
Ammonia N	10.15	9.85	9.95
Humin N	2.83	7.72	7.59
Arginine N	10.91	8.56	11.32
Histidine N	4.36	4.86	None
Lysine N	12.05	11.04	12.30
Cystine N	0.51	0.71	0.51
Amino N in filtrate from bases	55.43	52.02	58.61
Non-amino N in filtrate from bases	2.51	3.91	2.84
Total N regained	98.75	98.67	103.14

[1] The 3-gm. portion contained approximately 0.4550 gm. of nitrogen.

With this idea in mind the behavior of histidine in the presence of an ignited subsoil was tested, only three fractions being determined. A 0.5000-gm. sample of histidine di-hydrochloride[12] and 50 gm. of ignited subsoil were boiled in the presence of 100 c.c. of hydrochloric acid (sp. gr. 1.18) for 48 hours. The solution was diluted to 500 c.c. in a graduated flask and two 200-c.c. portions siphoned off and analyzed. The solution was deep straw color. The determinations for ammonia nitrogen gave negative results. The precipitate formed by calcium hydroxide was washed by decantation until no soluble nitrogen could be detected in the wash water and the precipitate Kjeldahled. This precipitate was bulky because of the presence of large amounts of ferric and aluminum hydroxides. The average nitrogen content of this fraction was only 0.0006 gm.

The filtrate from the calcium hydroxide precipitate was concentrated to a small volume and the entire solution used for the nitrogen determination. Sample I contained 0.0371 gm. in the filtrate, and Sample II, 0.0365 gm., making an average of 0.0368 gm.

[12] The histidine di-hydrochloride was prepared from dried blood as outlined by Abderhalden (1). Total nitrogen found was 18.42 per cent; calculated, 18.42 per cent.

The residual soil was practically colorless, and a determination indicated that it was nitrogen free. The volume occupied by the soil residue was 17.3 c.c. By calculation it was found that the total nitrogen regained in the original solution was 0.0903 gm., theoretical 0.0921 gm., or a recovery of 98.01 per cent.

Thus, practically all of the histidine was recovered in the filtrate from the calcium hydroxide precipitate, indicating that the above hypothesis was incorrect.

TABLE III

DIFFERENCE BETWEEN DUPLICATE ANALYSES (DUE TO EXPERIMENTAL ERRORS), THE DIFFERENCES APPARENTLY DUE TO THE ADDITION OF CARBOHYDRATE AND OF IGNITED SUBSOIL, AS WELL AS VAN SLYKE'S "MAXIMUM" AND "AVERAGE" DIFFERENCES TO BE EXPECTED

	Per cent difference between duplicates of fibrin alone [data of Gortner (10)]	Per cent difference between duplicates of fibrin and 3 times its weight of carbohydrate [data of Gortner (10)]	Per cent difference between duplicates of fibrin and 100 gm. of ignited subsoil	Average % difference between fibrin hydrolized alone and with 3 times its weight of carbohydrate [data of Gortner (10)]	Average % difference between fibrin hydrolyzed alone and in the presence of 100 gm. of ignited subsoil	Van Slyke's (46) experimental differences	
						"Maximum" per cent	"Average" per cent
Ammonia N	0.01	0.48	0.07	—0.30	—0.20	0.37	0.12
Humin N	0.11	0.25	0.10	+4.89	+4.76	0.39	0.20
Arginine N	0.73	0.86	3.49	—2.35	+0.41	1.27	0.73
Histidine N	0.07	0.66	None	+0.50	—4.36	2.14 [1](0.93)	0.79
Lysine N	0.08	0.11	1.47	—1.01	+0.25	1.23	0.61
Cystine N	0.10	0.00	....	+0.20	....	0.11	0.05
Amino N in filtrate from bases	2.12	2.15	0.49	—3.41	+3.18	1.60 [1](0.60)	0.63
Non-Amino N in filtrate from bases	0.27	0.40	3.23	+1.40	+0.33	1.20	0.68

[1] The figure in parentheses represents the second greatest difference between duplicates observed by Van Slyke (46).

Table III shows the differences between the duplicate determinations of the analysis of fibrin alone and in the presence of carbohydrate and of subsoil, and the differences apparently due to the addition of 100 gm. of ignited subsoil to the 3 gm. of fibrin. Van Slyk's (46) "maximum" and "average" differences to be expected between duplicate determinations are also given in the table for reference.

The table shows that the differences between the analyses of fibrin hydrolyzed alone and in the presence of ignited subsoil were, in most cases, within the maximum allowed by Van Slyke for experimental

error. The only differences which were *certainly* greater than experimental error were those of humin nitrogen, histidine nitrogen, and amino nitrogen in the filtrate from the bases. It was observed that practically the same error occurred with amino nitrogen in the filtrate from the bases when hydrolysis was carried out in the presence of carbohydrate.

From this analysis one can only draw the conclusion that even if *the organic matter of the soil consisted entirely of pure protein, one would not obtain the same nitrogen distribution by the Van Slyke analysis in the presence of soil that one would obtain in the absence of the soil,* or in other words, the presence of ignited mineral subsoil interferes with the Van Slyke analysis in much the same manner as carbohydrates [Gortner (10)].

TABLE IV

COMPARATIVE ANALYSES OF SPHAGNUM-COVERED PEAT HYDROLYZED ALONE AND IN THE PRESENCE OF NINE TIMES ITS WEIGHT OF A MINERAL SUBSOIL

	Grams Nitrogen			Apparent distribution of N in subsoil in per cent of total nitrogen
	Peat	Peat + Subsoil[1]	Increase (+) or Decrease (—)	
Total N	0.2000	0.2441	+0.0441	.....
Ammonia N	0.0466	0.0623	+0.0157	35.60
Humin N	0.0527	0.0658	+0.0131	29.71
Basic N	0.0195	0.0268	+0.0073	16.55
N set free as NH_3 by 50% KOH	0.0060	0.0100	+0.0040	9.07
N not set free by 50% KOH	0.0135	0.0168	+0.0033	7.48
Amino N of bases	0.0105	0.0178	+0.0073	16.55
Non-Amino N of bases	0.0090	0.0090	.....	....
N in filtrate from bases	0.0860	0.0951	+0.0091	20.64
Amino N in filtrate from bases	0.0776	0.0825	+0.0049	11.11
Non-Amino N in filtrate from bases	0.0084	0.0126	+0.0042	9.52
Total N regained	0.2048	0.2500	+0.0452	102.50

[1] Ninety gm. of subsoil contained 0.0441 gm. of soil nitrogen.

The humin nitrogen, its origin and significance. In such a discussion one must first consider the source of humin nitrogen in pure proteins.

Osborne and Jones (25) suggest that perhaps trytophane and histidine are responsible for the humin formation, basing their postulation on the fact that zein, which contains no trytophane and but little histidine, gives only small amounts of humin on hydrolysis.

Gortner and Blish (12) hydrolyzed zein in the presence of both trytophane and of histidine and found that a large part of the trytophane was converted into humin nitrogen, whereas none of the histidine was converted into humin but was all recoverable in the bases. It has been shown above that histidine is practically all recovered in the filtrate from the humin when it is hydrolyzed in the presence of an ignited mineral

subsoil. Histidine, therefore, may be eliminated as a factor in the formation of humin nitrogen in the soil. Gortner and Blish conclude that "in all probability the humin nitrogen of *protein* hydrolyses has its origin in the trytophane nucleus."

Gortner (10) has shown that the humin nitrogen is increased by the addition of carbohydrate material to protein, and suggests that this increase may be due to both physical and chemical causes.[13] He presents evidence to show that the action of carbohydrate is probably due to the furfural produced from the carbohydrate and shows that increasing quantities of furfural cause the humin nitrogen to increase steadily.

TABLE V

PERCENTAGES OF SOIL AND OF SOIL NITROGEN DISSOLVED BY HYDROLYZING THE DIFFERENT SOIL TYPES

Soil Type	Sample	Grams soil taken (dry basis)	Grams soil dissolved	Per cent soil dissolved	Grams nitrogen in soil	Grams nitrogen dissolved[1]	Per cent of dissolved nitrogen
Fargo clay loam.....	I	240.2	43.2	17.99	0.6005	0.4252	70.99
	II	240.2	43.2	.....	.....	.....	.....
Fargo silt loam.....	I	222.8	54.8	24.60	1.8336	1.4237	77.65
Carrington silt loam.	I	235.5	36.5	16.61	0.8738	0.6191	70.91
Hempstead silt loam.	I	242.3	32.3	13.33	0.6201	0.4395	70.87
	II	242.3	33.3	13.74	.....	0.4508	72.69
Prairie-covered loess.	I	230.3	42.3	18.37	0.6933	0.5260	75.91
	II	230.3	45.3	19.64	.....	0.4991	72.02
Forest-covered loess.	I	294.4	29.4	9.98	0.3768	0.2735	72.19
	II	294.4	32.4	11.11	.....	0.2511	66.65

[1] The figures in this column were obtained by subtracting the "insoluble humin nitrogen" remaining in the residual soil from the nitrogen figures obtained by multiplying the original weight of soil taken (dry basis) by the nitrogen content of the soil. These figures may or may not agree with the figures obtained in Kjeldahling a portion of the solution, as a result of experimental errors, and perhaps to errors introduced in using a uniform factor (2.6) for specific gravity. The figures in this column are free from any error of this sort.

Shmook (36) states that during the hydrolysis of his soils there separated on the walls of the condenser a substance violet blue in color, and that this appears during the hydrolysis of pure protein and is recognized as Liebermann's reaction for protein substances. The above conclusion in regard to the hydrolysis of a pure protein is incorrect, since no color appears on the neck of the flask or condenser in such an analysis. When

[13] Practically the same increase in humin nitrogen occurred when fibrin was hydrolyzed in the presence of a mineral subsoil. The humin in this case was not due to the presence of carbohydrate, since the soil had lost all of its organic matter by ignition.

furfural is heated alone with hydrochloric acid a characteristic colored substance is deposited on the condenser. It has been shown [Gortner (10)] that at the same time a polymerization (?) of furfural to humin takes places very rapidly. Our soils on hydrolysis gave a deposit on the condenser similar to that described by Shmook. The reaction indicates the presence of furfural, which is in turn formed from the carbohydrates in the soil.

The humin nitrogen of protein origin actually present in the hydrolyzed soil may easily be a very small part of the nitrogen found. It is evident that there must be many nitrogenous organic compounds present in the soil which have no relation to protein material, such as purine and pyrimidine bases, nitrogenous fats, and nitrogenous pigments, besides a number of other non-protein substances. It is certain that the humin nitrogen will be greatly changed by the presence of many of these compounds. The calcium hydroxide here drags down all the organic nitrogenous compounds which are soluble in dilute acids, but insoluble in hot water and dilute calcium hydroxide, together with the calcium salts of nitrogenous organic acids, the calcium salts of the purine and pyrimidine bases in addition to the humin formed from the protein material, and other organic compounds that are adsorbed, absorbed, occluded, or combined with the iron and aluminum hydroxides present.

From Table VI we find that from 3.26 to 9.21 per cent of the total nitrogen is precipitated by calcium hydroxide. This does not represent true humin nitrogen, since the calcium hydroxide precipitate does not contain any black colored substances formed by hydrolysis. The solution from which it is precipitated is colored only by ferric compounds; therefore, *the organic material in this precipitate must consist of colorless organic compounds adsorbed by or combined with the lime.* This portion of the nitrogen consists almost certainly of non-protein material. In all pure proteins the nitrogen retained in the calcium hydroxide precipitate is supposed to consist entirely of deeply colored substances. This study of the distribution of organic nitrogen in the soil has led to this new fraction, not previously reported. Certain of the analyses were carried out before the possible importance of this fraction was realized, but in most of the analyses this fraction is reported as "nitrogen retained by calcium hydroxide." Investigations as to the chemical nature of this fraction are highly desirable.

The *true humin nitrogen* of protein origin remains in the residual soil after hydrolysis. The amount of nitrogen in this fraction varies from 22.93 per cent to 28.27 per cent of the total nitrogen for the mineral soils studied. This represents more nearly the true humin nitrogen, in that the black coloring matter formed by acid hydrolysis remains in this fraction, but in addition we should also find here all organic nitrogenous

TABLE VI

AVERAGE NITROGEN DISTRIBUTION IN PER CENT OF THE TOTAL NITROGEN

	Sphagnum peat	Sphagnum peat + subsoil	Sphagnum peat + tin	1% HCl extract of sphagnum peat	Sphagnum peat soluble in 4% NaOH not pptd. by HCl	Sphagnum peat soluble in 4% NaOH and pptd. by HCl	Calcareous black peat	"Muck"	Fargo clay loam	Fargo silt loam	Carrington silt loam Sample I	Carrington silt loam Sample II	Hempstead silt loam	Prairie-covered loess	Forest-covered loess
Ammonia N	23.27	25.52	20.18	65.40	26.58	16.22	19.26	19.49	24.00	26.56	28.55	28.07	29.44	30.53	28.69
Insoluble humin N in soil	*	*	*	*	2.73	*	*	*	28.27	22.93	25.84	24.59	27.56	24.35	26.92
"Humin" N pptd. by $Ca(OH)_2$	*	*	*	*	8.97	*	*	*	9.21	[1]3.26	5.93	6.63	4.80	5.19	4.84
Total "humin" N	26.38	26.96	22.48	10.16	11.70	33.22	26.07	27.61	37.48	26.19	31.77	31.22	32.36	29.54	31.76
Total basic N	9.73	10.98	12.28	6.01	8.67	10.80	10.60	13.55	9.58	12.11	11.71	14.74	11.39	12.68	13.98
Basic N set free as NH_3 by 50% KOH	2.98	4.09	3.31	2.80	1.87	2.45	3.27	3.10	3.23	3.20	3.36	3.17	2.28	2.92	3.69
Basic N not set free as NH_3 by 50% KOH	6.75	6.89	8.97	3.21	6.80	8.35	7.33	10.45	6.35	8.91	8.35	11.57	9.10	9.76	10.29
Amino N of bases	5.26	7.29	6.86	4.11	4.28	6.08	6.35	9.13	6.44	7.52	8.04	7.99	7.44	7.48	7.57
Non-amino N of bases	4.47	3.69	5.41	1.90	4.39	4.72	4.25	4.42	3.14	4.59	3.67	6.75	3.95	5.20	6.41
N in filtrate from bases	43.00	38.96	46.40	20.64	53.69	41.08	42.40	38.81	32.71	35.14	25.47	28.25	28.10	28.80	27.21
Amino N in filtrate from bases	40.45	33.82	43.40	17.11	46.07	33.74	39.46	33.82	30.77	32.64	**	25.85	24.56	26.24	25.17
Non-amino N in filtrate from bases	2.55	5.14	2.99	3.53	7.62	7.34	2.94	4.99	1.95	2.50	.....	2.40	3.54	2.56	2.04
Total N regained	102.28	102.42	101.34	102.21	100.64	101.32	98.33	99.46	103.77	[2]100.00	97.50	102.28	101.29	101.56	101.64

* Not determined separately. ** Solution lost at this point. [1] By difference. [2] Calculated.

compounds insoluble in a fairly strong solution of hydrochloric acid, all of the nitrogen adsorbed by the carbohydrate humins, etc. Potter and Snyder (28) express surprise at the large proportion of nitrogen in this fraction, but when one considers the heterogeneous nature of the soil organic matter it is perhaps more surprising to find that over 60 per cent of the nitrogenous compounds are soluble in strong hydrochloric acid. Further study is necessary before the full significance and origin of this humin nitrogen can be thoroughly understood.

The effect of the quantity of acid used for the hydrolysis on the amount of nitrogen dissolved and the nitrogen distribution in soils. Throughout this investigation acid at least as strong as constant boiling hydrochloric acid was used for the hydrolysis, inasmuch as that is the strength ordinarily employed in the hydrolysis of proteins.

In the case of two soils, however, one of the duplicates was hydrolyzed in the presence of 1000 c.c. of concentrated acid to 250 gm. of soil, the other being hydrolyzed in the presence of 500 c.c. of constant boiling acid to 250 gm. of soil, in order to see if any noticeable differences would be observed between the resulting analyses. The two soils thus hydrolyzed were the prairie-covered loess and forest-covered loess.

The results show little difference between the duplicates. Table V shows that the larger volume of the stronger acid dissolved a greater per cent of the soil, because of the fact that more of the mineral constituents were soluble in acid of this concentration. At the same time, however, the amount of nitrogen extracted was less. It is perfectly obvious that sufficient acid was used in all experiments to secure uniform and maximum hydrolysis.

The percentage of soil nitrogen extracted by acid hydrolysis. Shorey (37) working with a single Hawaiian soil extracted 84.68 per cent of the total soil nitrogen by acid hydrolysis. Jodidi (17) working with 11 Iowa soils found from his studies a minimum of 68.90 per cent, a maximum of 83.94 per cent, and an average of 75.77 per cent; Lathrop and Brown (22) in 5 Pennsylvania soils found a minimum of 70.60 per cent, a maximum of 73.71 per cent, and an average of 71.78 per cent; Shmook (36), working with 4 Russian soils, found a minimum of 60.60 per cent in the Laterite soil, a maximum of 87.67 per cent in the Podzol soil, and an average of 68.33 per cent; Kelley (18), working with 9 soils of the Laterite class common to the Hawaiian Islands, found a minimum of 67.51 per cent, a maximum of 91.80 per cent, and an average of 82.17 per cent; and Potter and Snyder (28), in 7 Iowa soils, found a minimum of 68.68 per cent, a maximum of 76.47 per cent, and an average of 74.41 per cent.

The grand average of all of these 37 soils from widely different origin gives 75.91 per cent of the soil nitrogen in solution in the hydro-

chloric acid extract. In these studies there was found a minimum of 66.63 per cent, a maximum of 77.65 per cent, and an average of 72.19 per cent extracted by the acid.

These results indicate that the nitrogen of practically all soils, in so far as investigated, dissolves to about the same extent during acid hydrolysis.

A consideration of nitrogen distribution in different extracts from the sphagnum-covered peat. Nitrogen distribution was determined on extracts of a sphagnum-covered peat soluble in (a) 1 per cent hydrochloric acid, (b) 4 per cent sodium hydroxide and *not* precipitated by acidification, and (c) 4 per cent sodium hydroxide and precipitated by acidification with hydrochloric acid. Of the three extracts only the second approximates the distribution of nitrogen in a pure protein. The figures for the ammonia nitrogen are abnormally high in the hydrochloric acid extract.

The humin nitrogen is high in all the extracts, but is excessive in fraction (c). It is clear that carbohydrates from the soil must be present in all three fractions used, and must have some share in bringing the humin nitrogen up to such high figures. The nucleic acids [Shorey (39, 40)] would be found in the hydrochloric acid precipitate from the sodium hydroxide solution, and the purine and pyrimidine components of these nucleic acids, as well as the lecithins [Aso (2), Stoklasa (42)] and nitrogenous fats and nitrogenous acids would be precipitated with the true humin by the calcium hydroxide.

The basic nitrogen figures are not widely divergent, although there may be some significant differences.

The differences between the nitrogen in the filtrate from the bases is perhaps the most significant of all. An amino nitrogen of only 17.11 per cent in the filtrate from the bases such as is found in the hydrochloric acid extract, is far lower than has ever been obtained in an analysis of a pure protein and indicates that the nitrogen of this extract is essentially non-protein.

Unfortunately it was impossible to complete the corresponding analyses on the calcareous black peat, but the fractions obtained indicated a distribution similar to that of the sphagnum-covered peat.

General Conclusions in Regard to the Distribution of Soil Nitrogen in Different Soil Types

From a study of Table VI a great similarity is observed between the different analyses of different soil types. Practically the same deduction was made by Potter and Snyder (28) in their study of a single soil type under different fertilizer treatment.

We find that the nitrogen distribution in a soil is very uniform, whether in the same soil type under different fertilizer treatment, or in

different soil types. This is to be expected, for if we were to take at random 50 Van Slyke analyses of proteins and compare the average analysis with that of another 50 analyses, we should expect to find results agreeing closely with each other. This expectation should also hold true for the hydrolysate of soils, since in each soil are to be found many of the nitrogenous compounds contained in the plant and animal products that find their way to the soil together with their decomposition products. Since there is such a great variety of different nitrogenous substances in the soil, it stands to reason that the nitrogen distribution in soils is an *average* distribution, and as such should not be expected to vary widely from soil to soil.

Summary

This paper deals with a study of the nitrogen distribution, Van Slyke's method being used, in different soil types. Tables have been presented showing such distribution for the following materials:

a. Fibrin hydrolyzed in the presence of an ignited mineral subsoil, (together with data of fibrin hydrolyzed alone and in the presence of carbohydrates).

b. A calcareous black peat.

c. An acid, sphagnum-covered peat, hydrolyzed alone, in the presence of a mineral subsoil, and in the presence of stannous chloride.

d. An acid "muck" soil.

e. Seven samples of mineral surface soil representing the following soil types: Fargo clay loam, Fargo silt loam, Carrington silt loam (two samples from different glacial drifts), Hempstead silt loam, prairie-covered loess, and forest-covered loess.

f. Extracts of a sphagnum-covered peat soluble in (a) 1 per cent hydrochloric acid, (b) 4 per cent sodium hydroxide but precipitated by acid, and (c) 4 per cent sodium hydroxide and *not* precipitated by hydrochloric acid.

The following conclusions are evident:

1. The figures for the ammonia nitrogen in a protein analysis are not appreciably changed when the hydrolysis is carried out in the presence of an ignited mineral soil equal to twenty times the weight of the protein material.

2. The "humin" nitrogen was greatly increased by hydrolysis in the presence of ignited mineral soil. The histidine fraction entirely disappeared.

3. Attention is called to the fact that the analysis of a pure protein in the presence of an ignited mineral soil does not give reliable results for the different fractions. Therefore, the figures obtained for the nitrogen distribution in soils are of value only when used for purposes of comparison. Such data should not be compared with analyses of pure proteins.

4. Since practically all mineral soils give furfural on treatment with acid it is very likely that a very considerable amount of the total humin nitrogen found is due to the presence of carbohydrates in the soil, which give rise to furfural during hydrolysis. This may combine with certain of the nitrogenous compounds and cause an increase in the humin nitrogen, as well as adsorb or occlude nitrogenous compounds in the "humin" formed from furfural by polymerization.

5. This investigation of the distribution of organic nitrogen in the soil has indicated a new fraction which should be recorded separately. This is the fraction of nitrogen removed from a colorless solution by calcium, iron, and aluminium hydroxides on the addition of calcium hydroxide. The nitrogen retained in this fraction must consist almost entirely of material of non-protein origin, since the organic substances in this precipitate have been shown to be *colorless* organic compounds adsorbed by or combined with the metallic hydroxides. This fraction has been reported as nitrogen precipitated by calcium hydroxide.

6. The *true* humin nitrogen remains in the residual soil after hydrolysis, but in addition non-humin nitrogenous compounds are also retained in this fraction.

7. The strength and volume of the hydrochloric acid used in hydrolysis has little effect on the nitrogen distribution of the hydrolysate, provided acid as strong as constant boiling acid is used, in the proportion of at least two parts of acid to one of soil.

8. Results gained from a study of different soils indicate that the organic nitrogen dissolves during hydrolysis, to almost the same extent regardless of the origin and nature of the soil.

9. Some very interesting figures are found in the comparison of the different extracts from sphagnum-covered peat. The portion soluble in sodium hydroxide and *not* precipitated by hydrochloric acid, gives a nitrogen distribution approximating very closely that of a normal plant protein. The nitrogen dissolving in the preliminary hydrochloric acid leaching shows a nitrogen distribution which is *certainly* not due exclusively to protein materials, *e. g.*, an ammonia nitrogen percentage of 65.40 and amino nitrogen in filtrate from bases of 17.11 per cent.

10. The most significant fact brought out by this study is that the organic nitrogen distribution in *different soil types* is very uniform. This is to be expected, since it has been pointed out that the nitrogen distribution in soils is an *average* distribution of all the plant and animal nitrogenous products that find their way into the soil.

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SOIL CONSTITUENTS WHICH INHIBIT THE ACTION OF PLANT TOXINS[1]

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Introduction

Along certain lines, the subject of plant toxins has been studied extensively by many investigators. A widely discussed theory of soil infertility has been advanced by the Federal Bureau of Soils (6, 18, 25, 27, 47, 48) which ascribes the infertility of soils, not to inadequate supply of plant-food but to the presence of complex organic substances which are toxic to plants. According to this theory the beneficial effects of fertilizers are due mainly to the counteraction of these toxins. In consideration of this theory it is readily apparent that the relation of all the soil constituents to these toxic substances is of prime importance. One of the first questions which should present itself to the soils investigator in this connection is: Are there constituents, common to our agricultural soils, which, because of their specific or peculiar nature, may inhibit the action of these plant toxins in a chemical as well as physical way? The investigation reported in this paper was undertaken for the purpose of studying this question.

The term "plant toxin" as used in this discussion refers to those substances, which, at concentrations considerably below the osmotic equivalent of the cell-sap, are injurious to living plant protoplasm. This definition includes both inorganic and organic compounds, which may be either acidic, basic, or neutral in character.

Historical

As early as 1893, Nägeli (23) discovered the beneficial effects of the addition of insoluble particles of matter to distilled water which was toxic to algae on account of its content of traces of copper. By adding crushed graphite, shredded filter paper, paraffin shavings, or other insoluble substances, in a finely divided state, he was able to inhibit entirely the toxic effects of the copper.

Most of the work done in the past with the higher plants has been done with solution cultures. Heald (13), in 1896, found acetic acid to be

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much less toxic to corn than were the common mineral acids, such as hydrochloric, sulfuric, nitric, and hydrobromic. The investigations of Kahlenberg and Mehl (19) indicate that practically the same order of toxicity is obtained when fishes (rock-bass) are used instead of corn seedlings.

The results obtained by Cameron and Breazeale (7) also indicate that the common mineral acids are more toxic to corn than are the common organic acids. But, on the other hand, the effect of the latter was somewhat more pronounced on wheat. With clover, they all seemed equally toxic.

By the addition of a corresponding potassium or calcium salt to the solution of a free acid, Cameron and Breazeale (7) were enabled to lessen greatly the toxicity of the latter. The ameliorating effect of the calcium salts was very striking and much more pronounced than that of the potassium salts. As the concentration of free acid present in each case was not lowered and the total concentration of the solution was raised, this beneficial effect is rather difficult of explanation except possibly on the basis of some change in the permeability of the osmotic membranes of the cell.

Harter (12) showed that the toxicity varies with the variety of the plant used, some varieties of wheat being more susceptible to certain salts than others. Thus, to make results comparable, the same variety of the same plant must be used throughout the experimental work.

That the presence of quartz sand reduces the toxicity of many solutions has been proved by the work of many investigators. Dandeno (8) found that the finer the sand used, the greater was the reduction of the toxic effect of copper sulfate solution. His results were corroborated by the experiments of Jensen (15) with sulfuric acid.

True and Oglevee (42) reduced the toxicity to *lupinus albus* of solutions of silver nitrate and copper sulfate by the addition of such insoluble substances as quartz sand, powdered glass, shredded filter paper, and starch grains. However, with very weak poisons, as thymol and resorcinol, the insoluble material had no beneficial effect. This is in accord with the generally accepted theory that the ameliorating effects in solution cultures are due to the adsorption of some of the toxic material out of solution, by the insoluble particles added. The amount adsorbed from a very dilute solution,—*e. g.*, a toxic solution of copper sulfate containing 1 gm. mol. per 30,000 to 50,000 liters, would be proportionately great, whereas that adsorbed from a more concentrated solution,—*e. g.*, a toxic solution of resorcinal containing 1 gm. mol. in less than 200 liters, would be but a very small percentage of the total amount present.

Using maize seedlings and sulfuric acid solution, Breazeale (4) obtained results contradicting those of True and Oglevee (42); no increased growth was observed in seedlings grown when quartz sand, quartz

flour, filter paper, or paraffin shavings were added to the solutions. With copper sulfate solutions, however, carbon black did improve them as mediums for plant growth.

Jensen's results (15), on the other hand, fully agree with those of True and Oglevee (42). Quartz flour reduced the toxicity almost invariably except with phenol and alcohol which, like resorcinol, are very weak poisons to plants. The effectiveness of quartz flour in reducing the toxic effect was most marked with nickel, less with silver, zinc, copper, iron, and lead solutions, in the order given, and no reduction whatever was apparent in phenol or alcohol solutions.

The toxic effects of arsenical solutions have been studied by numerous investigators, some of which are mentioned here. Nobbe, Baessler and Will (24) found that in water cultures, arsenious oxide was very poisonous to buckwheat, oats, maize and alder.

Knop (22) studied the relative toxicity of arsenious and arsenic acids to maize plants. His work showed the former to be more toxic.

Stoklasa (40, 41) corroborated Knop's work showing the greater toxic effects of arsenious acid and arsenites in comparison with arsenic acid and arsenates. He states that 1/100,000 gm. mol. wt. arsenious acid per liter causes definite trouble in plants, while 1/1000 gm. mol. wt. arsenic acid per liter first shows noticeable toxicity. Young barley seedlings were killed in 46 hours when placed in a 1/10,000 gm. mol. wt. arsenious acid solution, while it took 24.5 days to kill barley seedlings in a 1/1000 gm. mol. wt. (10 times as concentrated) solution of arsenic acid. In sand cultures, Stoklasa (41) got analogous results.

Experiments at Rothamsted (5) with barley also indicate a much greater toxicity with arsenious than with arsenic acid. Sodium arsenite, in nutrient solution cultures, showed a depressing effect on barley even at a dilution of N/250,000,000, but as strong a solution as N/100,000 concentration of arsenic acid apparently had no toxic effect.

While other experimenters did most of their work with mineral toxins, the investigators of the United States Bureau of Soils (25, 26, 29, 30, 31, 32, 33, 35, 36, 37) isolated many organic compounds from infertile soils, and studied the effects of these compounds in water solution on plant seedlings, principally wheat seedlings. Some compounds, as guanidine, dihydroxystearic acid, picoline carboxylic acid, vanillin, and salicylic aldehyde were found to be toxic to plants in water solution, while some others were not. As a result of their investigations, Whitney and Cameron arrived at the conclusion that the infertility of many soils is due largely to the presence of these and other organic toxins, which probably owe their origin to plant excretions (6, 17, 18, 27, 47) as well as to decomposition of plant residues. Schreiner and Reed (27) presented considerable experimental evidence in an attempt to show that the beneficial effects of fertilizer salts in enhancing the yields on many poor soils, is not due to the increased amount of available plant-food given to these soils,

but rather to the power of these fertilizers in counteracting the effects of the organic soil toxins. Whitney and Cameron (47) presented much data endeavoring to show that increasing the amount of plant-food by the addition of fertilizers has practically nothing to do with the permanent fertility of the soil.

Their work, however, is not in harmony with that of King (21) who, while chief of the Division of Soil Management of United States Bureau of Soils, investigated the relation of amount of water-soluble salts present in soils to the crop yields. He found that where the yields were notably different, considerably more water-soluble salts could be obtained from the more productive soils.

Extensive investigations at the Woburn Experimental Fruit Farm (2, 3) indicate that fruit trees may be greatly injured by toxic substances arising from a grass sod.

Although previous investigators apparently believed that physical processes are largely responsible in counteracting the effects of toxic substances that may be present in cultures or the soil, nevertheless, many of their results indicate that chemical processes may play an important rôle. Livingston (17) found that all the poor soils studied by him, as well as the aqueous extracts of these soils, were very considerably improved by the addition of calcium carbonate. These results are in accord with those of Breazeale (4).

A glance through Breazeale's data reveals the fact that the effects of calcium carbonate and ferric hydrate (chemical agents) are much more pronounced than those of carbon black and quartz flour (physical adsorptive agents). Ferric hydroxide, of course, also acts as a physical adsorptive agent.

Davidson's investigation (9) of the toxic properties of vanillin and cumarin, led him to the conclusion that his experiments would hardly lend much support to the assumption that the presence in the soil of organic substances toxic in water cultures is a factor of considerable importance under field conditions, when the other factors of plant growth are normally good.

Fraps (10), at Texas, also did considerable work with vanillin and cumarin in soil cultures. Under normal soil conditions the toxins were rapidly oxidized, a considerable proportion disappearing in 2 weeks and little remaining at the end of his experiments. Pyrogallic acid and carbon black did not increase crop yields on soils to which vanillin had been added. Livingston (18) had found a very pronounced beneficial effect when these substances were added to the water extracts of poor soils. In Fraps' experiments, the addition of acid phosphate and other fertilizer salts to the soils almost invariably increased crop yields. Consequently, Fraps stated that the poor soils studied by him needed the plant-food supplied by the fertilizers, and the action of the fertilizer is to supply plant-

food and not to overcome toxic substances. He found little evidence that fertilizers directly overcome the injurious action of cumarin or vanillin.

The toxic properties of guanidine to plants were studied by Kawakita (20). Schreiner and Skinner (34), working with guanidine carbonate, found it to be very poisonous to wheat seedlings grown in solution cultures. Bleached spots appeared on the leaves of the affected plants causing an appearance much like that due to a plant disease.

Schreiner and Skinner (36) also carried on an extensive investigation of the poisonous effects of salicylic aldehyde. They obtained results showing that calcium carbonate decreased the toxicity to wheat seedlings of solutions containing this compound. The roots of the plants in cultures containing salicylic aldehyde were especially improved in cultures containing calcium carbonate. Salicylic aldehyde was isolated from soils varying in texture from very fine sandy loams to clays. Of the garden soils containing this substance, three were acid, one was alkaline and one was neutral. Of the field soils in which it was found, ten were acid, one alkaline, and one neutral. Salicylic aldehyde had a pronounced depressing effect on cowpeas, string beans, and garden peas which were grown on a heavy, silty, clay loam, low in organic matter, to which the toxic agent was applied at the rate of 105 pounds per acre in 3 applications.

Skinner's investigations with vanillin (38) showed that this organic soil constituent depressed the yield of wheat, when grown in pots on certain poor soils, but that it did not affect the production on a good soil. Even on an unproductive sandy loam 100 parts per million of vanillin had no effect, while 200 parts per million decreased the yield but slightly. In a field experiment on an acid silty clay loam soil, vanillin was applied at the rate of 284 pounds per acre in 4 applications during a period of 36 days. Crop yields with several crops were reduced from that of control plots, from 20 to 69 per cent.

The work of Upson and Powell (45) indicates that vanillin is not appreciably toxic to wheat plants on a black silt loam of good texture even when 1000 parts of vanillin per million parts of soil are present. These investigators also studied the toxic action of salicylic aldehyde, which in pot tests was found to depress the growth of corn 24 per cent with a soil and 60 per cent with quartz sand. Using the same soil as with vanillin, they obtained results showing that the toxic effect on wheat, even in a concentration of 500 parts per million was practically negligible.

Recently Skinner and Noll (39) reported the results of field investigations regarding the toxic effects of vanillin and salicylic aldehyde on cowpeas. On an unproductive acid soil the toxic effects of both compounds were overcome by liming. In the limed soil both compounds disappeared after several months, while in the unlimed soil they were still present. On a productive soil only slight toxic effects were noted, which disappeared on adding fertilizer and lime. After several months both compounds disappeared in the productive soil.

In a recent report of extensive investigations on the effects of certain organic compounds on plant growth, Funchess (11) of Alabama states that a normal soil can apparently dispose of enormous quantities of organic compounds through physical, chemical, and biochemical action.

Experimental

From the references given, it seems that there are certain soil constituents which inhibit the action of plant toxins. The object of the present experimental work was, first, to determine what some of these soil constituent might be, and second, to determine whether their action is mainly physical, chemical, or both.

Materials Used

Most of the work was carried on in artificial soil cultures, contained in glass tumblers of about 180 c.c. capacity. Some experiments with field soils were conducted in 1-gallon earthenware jars.

In the preparation of the artificial soil cultures, several insoluble materials were used as follows: quartz sand, quartz flour, kaolin, and Superior red clay soil. The quartz sand was a natural unsifted sand analyzing 99.13 per cent silica. The quartz flour, which contained from 2 to 3 per cent of impurities, consisted of very finely ground material which had been passed through a 100-mesh sieve. It was obtained from a quartzite crusher. The kaolin used was also 100-meshed material; it came from Dry Branch, Ga. The Superior red clay soil employed for these experiments gave a strong acid reaction to various tests. It, also, was passed through a 100-mesh sieve before being used.

For the natural soil cultures, two acid soils, Plainfield sand and Wabash silt loam, were employed. The Plainfield sand, from Sparta, Wis., was a loose, strongly acid, sandy soil very deficient in fertility and responding readily to fertilizer treatment. The Wabash silt loam, which came from West Salem, Wis., was quite acid in reaction, but contained a very plentiful supply of fertility elements.

The chemicals employed, both for nutrient and toxic purposes, were commercial C. P. grade, except in the case of the guanidine carbonate. This compound was prepared in the laboratory in the usual manner (46) by the distillation of ammonium thiocyanate and subsequent treatment with potassium carbonate. The guanidine carbonate thus prepared was then purified by repeated recrystallization from alcohol and water.

A nutrient solution (5) was applied to all artificial soil cultures. It contained the following per liter:

$NaNO_3$	0.5	gm.
KNO_3	0.2	gm.
KH_2PO_4	0.1	gm.
$CaSO_4$	0.1	gm.
$MgSO_4$	0.1	gm.
NaCl	0.1	gm.
$FeCl_3$	0.04	gm.

The calcium carbonate used in the artificial soil cultures was C. P. precipitated material, but that added to the soil cultures was commercial 100-meshed ground limestone.

Preparation and Care of Artificial Soil Cultures

These always consisted of three series. To each tumbler 250 gm. of insoluble material was added. There was used per tumbler in Series I 250 gm. of quartz sand alone; in Series II, 225 gm. of sand and 25 gm. of quartz flour; and in Series III, 225 gm. of sand and 25 gm. of pure kaolin. In Experiment VIII, 25 gm. of Superior red clay soil was substituted for the kaolin in Series III of each set. In some experiments a second set of three series was set up, to each pot of which 1 gm. of calcium carbonate was added; otherwise this set was identical with the first. The quartz flour, kaolin, Superior clay, and calcium carbonate wherever used, were mixed thoroughly in the dry condition with the quartz sand on a rubber mixing cloth. The concentrations of toxins in Series II and III were always the same as those applied to Series I.

In the following description the concentrations of the inorganic toxins will be given in terms of normality, but those of guanidine carbonate will be expressed as parts per million of solution.

The total moisture content maintained in each pot in all artificial soil cultures was 13 per cent, or 32.5 c.c. It was recognized that perhaps for one reason somewhat more water should have been added to the kaolin cultures for optimum growing conditions, due to their greater water-holding capacity. Nevertheless, it was thought best to use the same quantity of water throughout in order that, not only the same concentration of toxic agent, but also the same quantity be present in each corresponding pot of the three series, thus giving nearest possible comparable results.

In all artificial soil cultures, the plants used were young seedlings of Blue Stem wheat. The wheat seeds were germinated between filter paper which was packed in wet quartz sand, until the radicles were about one-fourth inch long. Then they were carefully transferred to the cultures to which had been added the nutrient and toxic solutions in proper amount and in which the total moisture content had been brought up to 13 per cent. The cultures were then placed in the greenhouse and kept at approximately standard moisture content by the addition of distilled water from time to time as needed.

Natural Soil Cultures

For the Plainfield sand cultures, 6800 gm. of the air-dry soil were weighed into each earthenware pot. Two series of cultures were set up, the second identical with the first except that to each pot of the second enough ground limestone was added and mixed to neutralize the active acidity of the soil. To desired cultures, nutrients were applied in solution as follows: 0.5 gm. of potassium nitrate and 0.5 gm. of disodium hydrogen phosphate per pot. The moisture content of each culture was main-

tained at 14 per cent as that had been found to produce about optimum moisture conditions for plants growing in this soil.

In the case of the Wabash silt loam, each pot contained 4600 gm. of the air-dry soil. As with the Plainfield sand, two series of cultures were set up, one unlimed and the other limed. To desired cultures, the same amounts of nutrients were applied in solution per pot as with the Plainfield sand cultures. The moisture content was maintained at 30 per cent.

In each natural soil culture, 25 plump wheat seeds were planted. After the plants were well up, they were thinned out to 20 per pot.

Experiments with Copper Salts as the Toxic Agents in Artificial Soils

Experiment I.—The following concentrations of copper sulfate were used: N/300, N/200, N/100, N/50, N/10 and a control containing only the nutrients in solution. After the plants had grown for 9 days, the average length of plant for each pot was determined. The results are given in Table I. In the quartz sand cultures the leaves of the plants

TABLE I

THE TOXICITY OF COPPER SULFATE TO WHEAT PLANTS

(Average height of plants in inches)

Concentration of Toxin in Solution	Composition of Culture Medium		
	Series I 250 gm. Quartz Sand	Series II 225 gm. Sand and 25 gm. Quartz Flour	Series III 225 gm. Sand and 25 gm. Kaolin
Control	10.0	7.0	6.5
N/300	3.5	4.5	4.5
N/200	3.0	4.0	4.0
N/100	2.0	2.5	2.5
N/50	1.5	1.5	1.5
N/10	No growth	No growth	No growth

were very narrow, but in the cultures of Series II and III they were of normal width. In the case of the controls the plants started more quickly in Series I than in the other series and hence made a taller growth. A set of duplicate cultures showed the same results as those given in Table I.

Both the quartz flour and the kaolin had a slightly beneficial effect in the more dilute concentrations compared with the quartz sand series. This is as it should be if adsorption came into play, as it undoubtedly did, for the addition of quartz flour and kaolin greatly increased the surface exposed to the solution.

Experiment II.—A new set of cultures with copper sulfate was started. A second set was added, identical with the first in every way except that 1 gm. of calcium carbonate was applied to each culture. Assuming 2,000,000 pounds as the weight of the surface 8 inches of an acre of ordinary loam soil, this application of calcium carbonate is equivalent to 8000 pounds per acre. The plants were grown for 14 days, when they were photographed and then harvested. Figures 1 and 2 in Plate I show

how remarkable was the general ameliorating effect of the calcium carbonate, as is also shown by the dry weights recorded in Table II.

TABLE II

THE EFFECT OF CALCIUM CARBONATE IN INHIBITING THE TOXICITY OF COPPER SULFATE TO WHEAT PLANTS

Culture Medium	Concentration of Toxin in Solution	Dry Weight of Tops	
		Unlimed gm.	Limed gm.
250 gm. quartz sand	Control	0.056	0.047
	N/300	0.038	0.068
	N/200	0.036	0.045
	N/100	0.034	0.060
	N/50	0.016	0.067
	N/10	No growth	0.054
225 gm. quartz sand and 25 gm. quartz flour.....	Control	0.054	0.059
	N/300	0.053	0.072
	N/200	0.041	0.064
	N/100	0.031	0.066
	N/50	0.012	0.068
	N/10	No growth	0.048
225 gm. quartz sand and 25 gm. kaolin	Control	0.050	0.062
	N/300	0.036	0.056
	N/200	0.028	0.064
	N/100	0.026	0.069
	N/50	0.003	0.054
	N/10	No growth	0.060

These results indicate a strong contrast in the relative effectiveness of physical and chemical processes in the reduction of the toxicity of copper sulfate to wheat plants. Calcium carbonate, evidently due to a reaction with the copper sulfate, seems to have entirely destroyed the effect of the latter. The reaction probably takes place as follows:

$$CuSO_4 + CaCO_3 = CuCO_3 + CaSO_4$$

Since the copper carbonate formed is quite insoluble, the reaction goes practically to completion, with a resultant removal of the copper from the soil solution. In a concentration of N/10 copper sulfate, where there was no growth even in the quartz flour or kaolin cultures, the addition of calcium carbonate resulted in a top growth as good as that of the control. The root growth, however, was somewhat depressed. In the lesser concentrations, which had been decidely toxic without calcium carbonate, there was a marked stimulating effect when calcium carbonate was present, especially in the quartz sand and quartz flour cultures. In this experiment the unlimed kaolin cultures made a poorer growth than the unlimed quartz sand cultures.

Experiment III.—In this experiment copper nitrate was used in order to furnish a comparison of the action of copper salts of different acids. The three highest concentrations of the previous experiments were chosen for this work—*i. e.*, N/100, N/50, and N/10. A control was used for each series as usual. Two sets of cultures, one unlimed and the

other limed, were prepared and the wheat seedlings planted. At the end of 9 days, the roots were carefully washed out and some of the plants photographed. Plate II (fig. 1) furnishes a good summary of the results. The effect of the calcium carbonate was almost as striking as with copper sulfate and is undoubtedly to be explained on the same basis. The growth in the kaolin cultures was somewhat better than in the sand or quartz flour cultures.

Experiments with Sodium Arsenite as the Toxic Agent in Artificial Soils

Experiment IV.—Cultures with quartz sand alone, quartz sand plus quartz flour, and with quartz sand plus kaolin were set up as in Experiment I. The solutions of sodium arsenite used were necessarily much less concentrated than those of copper sulfate. They were as follows: N/6400, N/3200, N/1600, N/800, N/400 and a control containing only the nutrients in solution. The wheat plants were allowed to grow for 9 days when the average length of the plants was determined. The results are given in Table III.

TABLE III

THE TOXICITY OF SODIUM ARSENITE TO WHEAT PLANTS
(Average height of plants in inches)

Concentration of Toxin in Solution	Composition of Culture Medium		
	Series I 250 gm. Quartz Sand	Series II 225 gm. Sand and 25 gm. Quartz Flour	Series III 225 gm. Sand and 25 gm. Kaolin
Control	6.0	7.5	6.5
N/6400	5.5	7.5	5.5
N/3200	4.5	7.0	4.5
N/1600	3.0	7.5	3.5
N/800	2.0	6.5	1.0
N/400	1.0	6.0	No growth

The toxic effects in the quartz sand and in the kaolin cultures were marked and approximately the same, but in the quartz flour cultures there was very little toxic effect shown. This could not possibly be explained on the basis of differences in the relative adsorptive powers of the materials used, for from that view point the kaolin cultures, with the high adsorptive capacity due to the large surface exposure, should have given as good results as the quartz flour cultures, or better. It seems more probable, therefore, that certain impurities, known to be present in the quartz flour, acted as catalytic agents in the oxidation of sodium arsenite to sodium arsenate, which is much less poisonous than the arsenite, as has been shown by the work of other investigators (5, 22, 40, 41).

Experiment V.—Instead of preparing new cultures for this experiment, the old cultures of Experiment IV were used and replanted with wheat seedlings. The pots had stood in the greenhouse without plants from December 16 to January 8.

Whereas in Experiment IV the seedlings made no growth in the N/400 kaolin culture and practically none in the corresponding quartz sand culture (see Table III), the replanted seedlings made a good growth in these cultures. These results lend weight to the possible explanation offered for the good results obtained in Experiment IV with quartz flour. The sodium arsenite in the quartz sand and in the kaolin cultures had probably slowly oxidized on standing, thus reducing the toxicity of the soil solution, while in the quartz flour cultures this oxidation went on even more rapidly, due to the presence of impurities which may have acted as catalysts.

Experiment VI.—Cultures were set up as in preceding experiments, but only three concentrations of sodium arsenite were used, *i. e.*, N/3200, N/800 and N/200. As usual, there was a control for each series. Two sets of culture series were used, one unlimed and the other limed. At the end of 10 days' growth, the roots were carefully washed out and some of the plants photographed. Plate II (fig 2) gives a good idea of the results.

As is seen from the figure, the arsenite was very toxic to wheat seedlings in pure quartz sand, and moreover, the addition of calcium carbonate had practically no ameliorating effect. While quartz flour, as in Experiments IV and V, exerted a markedly beneficial effect, the addition of calcium carbonate did not enhance that effect. With kaolin, however, the calcium carbonate produced a decided increase over the growth in the cultures without it. This can possibly be explained by a chemical reaction in which small amounts of manganese or other activating chemical elements may have been set free from the kaolin by replacement with calcium, and thus as catalysts accelerated the oxidation of the sodium arsenite to the higher salt, resulting in a reduction of toxicity. Here, as in the other experiments, the evidence indicates that chemical reactions were much more important than adsorption in lessening the toxicity of certain poisons to plants.

Experiments with Guanidine Carbonate as the Toxic Agent in Artificial Soils

Experiment VII.—Two sets of the usual culture series were prepared, one set being unlimed and the other limed. The concentrations of guanidine carbonate solution used in each series were as follows: 25, 50, 200, 500, and 1000 parts per million of solution, and a control containing only nutrients in solution. Wheat seedlings were planted and allowed to grow for 12 days, when the roots were carefully washed out and the plants photographed. A good conception of the results can be obtained from Plates III, IV and V (fig. 1).

The results obtained with quartz flour cultures were practically identical with those of the quartz sand cultures and are therefore not shown in detail.

In the cultures containing 200 or more parts per million of guanidine carbonate, the poisonous effects of the toxic agent became evident after 3

to 5 days. On the leaves of the plants grown in these cultures bleached spots appeared. These spots spread until they coalesced, causing the leaf to wilt and die. The bleached effect became apparent earliest near the juncture of the leaf with the stem, thus destroying the turgidity and causing the leaf to droop. These effects corresponded exactly to those described by Schreiner and Skinner (34). The root growth, as well as the top growth, of the affected plants was strongly inhibited, as is shown by the figures referred to above. The outstanding feature of the results obtained was the very beneficial effect of the kaolin, especially on the root growth. Plants grown in kaolin cultures containing 1000 parts per million of guanidine carbonate showed as good a root growth as plants grown in quartz sand or quartz flour cultures containing only 200 parts per million, while seedlings grown in sand or quartz flour cultures containing 1000 parts per million made no root growth and practically no top growth. Figures 1 and 2 in Plate III bring out clearly the remarkable ameliorating effect of the kaolin, especially on root development. Even with cultures to which no toxin was added, the kaolin had a very favorable effect on root development. In these cases the kaolin may have inhibited the slight action of small amounts of toxins which may possibly have been given off by the plant roots themselves. A second outstanding feature of the results was the lack of any beneficial effect due to the addition of calcium carbonate; in fact, it exerted a marked depressing effect in the kaolin culture containing 1000 parts per million of guanidine carbonate.

The quartz flour cultures, with their greater adsorbing surface, provided no better conditions for plant growth than the quartz sand cultures. Thus, to find the explanation of the observed results, it is necessary to consider carefully the chemical constitution and possible interreaction of the materials dealt with, for it is plainly evident that physical adsorptive phenomena are inadequate for this explanation.

Guanidine (14) is a strong base whose structural formula is written thus:

$$C(=NH)(NH_2)(NH_2)$$

Investigators (16), have obtained results showing kaolin to be acid in nature. Asch and Asch (1), who consider kaolin to be an acid, believe that it polymerizes to form either of two isomeric kaolinic acids which have different physical and chemical properties. According to their theory, one isomer may pass over into the other under suitable conditions.

If one accepts the idea that kaolin is an acid, the experimental results obtained are very easily interpreted. Thus the strong base guanidine splits off from the very weak acid carbon dioxide and combines with the kaolinic acid to form an insoluble compound. The guanidine is thus removed from solution, at least in part, and hence its effect on plants is greatly lessened. A further application of this idea of the acid nature of

kaolin serves admirably to explain the detrimental effect of the calcium carbonate when added to the kaolin cultures. The calcium competes strongly with the guanidine for the kaolinic acid. As a result, less guanidine is removed from the soil solution and hence its toxicity is reduced but little. That the kaolin need not react acid to litmus in order to bring about this beneficial effect is shown very clearly in this experiment, for the kaolin used gave no reaction when tested with litmus paper. However, the base guanidine apparently was strong enough to replace bases already combined with the kaolin. It is also possible that in contact with a strong soluble base like guanidine, the kaolin, at least partly, changes to a more active acidic form. When the kaolin is in an active acidic state, its beneficial effect, as would be expected, is much more marked, as will be indicated in the following experiment.

Experiment VIII.—This experiment was undertaken for the purpose of comparing the effect of pure kaolin with the effect of impure kaolin or clay of a natural soil. For this purpose 25-gm. samples of acid Superior red clay were added to each culture of Series III of both the unlimed and limed sets instead of adding the 25 gm. of pure kaolin used in Experiment VII. As is well known, a clay soil usually contains a very large percentage of kaolin, for it is derived largely from minerals of the same nature as those from which kaolin originates. In this experiment only the three highest concentrations of guanidine carbonate were employed, *i. e.*, 200, 500, and 1000 parts per million. As usual, a control, containing only the nutrients in solution was set up for each series.

The seedlings were grown for 10 days when the roots were washed out as usual and some of the plants photographed. The characteristic effects of guanidine poisoning had again become apparent after 4 or 5 days. The results are partly given in Plate V (fig. 2), and corresponded with those of Experiment VII except that the addition of calcium carbonate had no inhibiting effect on the clay cultures, while it had a decided inhibiting effect on the plants grown in the kaolin culture containing 1000 parts per million of guanidine carbonate. A comparison of plant No. 13 in Plate V (fig. 2) grown in a Superior acid clay culture with the corresponding one—No. 29, in Plate III (fig. 2), grown in a kaolin culture, shows the ameliorating effect of the acid clay to be considerably more marked than that of the kaolin. This accords well with the theory of chemical reaction. Since the Superior clay was strongly acid to litmus while the kaolin was neutral to this indicator, it is only logical to infer that the former would combine more readily and completely with the base guanidine, than would the latter. This, as the results indicate, is also true when considerable calcium carbonate is present.

In this experiment, there was but a slight ameliorating effect due to the presence of the very finely ground quartz flour, thus further supporting the view that adsorption plays but little part in counteracting the toxicity of guanidine carbonate.

Experiments with Vanillin as the Toxic Agent in Natural Soils

Experiment IX.—Two series of pot cultures, each pot containing 6800 gm. of air-dry soil, were provided for. Plainfield sand, a soil low in fertility and strongly acid in reaction, was used. Series I was unlimed, but to each pot of Series II enough ground limestone was added to neutralize the active acidity. Vanillin was applied to some of the pots at the rate of 500 parts per 2,000,000 parts of soil or approximately 500 pounds per acre. To the desired pots nutrients were added in the form of potassium nitrate and disodium hydrogen phosphate. Controls were included in each series. All work was done in duplicate. Wheat was planted and thinned to 20 plants per pot. After 39 days' growth, photographs were taken and after 42 days' growth the crops were cut and dried. Figure 3 in Plate I shows that the addition of limestone and fertilizers was decidedly beneficial to the wheat plants grown on the pots to which vanillin had been added. Table IV gives the dry weights of the tops and further bears out this beneficial effect.

TABLE IV

EFFECTS OF VANILLIN ON THE GROWTH OF WHEAT ON AN INFERTILE, ACID SAND

Treatment	Dry Weight of Tops in gm.			
	Number of Pot	Series I Unlimed	Number of Pot	Series II Limed
Blank	1	1.006	9	1.223
Blank	2	0.896	10	1.405
Vanillin	3	0.484	11	1.405
Vanillin	4	0.501	12	1.199
Nutrients	5	1.772	13	2.373
Nutrients	6	1.601	14	2.531
Nutrients and Vanillin.	7	1.389	15	2.429
Nutrients and Vanillin.	8	1.565	16	2.566

In studying the results in this table one notes that vanillin was decidedly toxic on this poor sandy soil when neither nutrients nor limestone were added. However, where nutrients were applied, the poisonous action of the vanillin was greatly lessened. The same was true, but in a more marked degree, where limestone alone was added. When nutrients to increase the fertility, and limestone to neutralize the acidity, were both applied, the toxic effect entirely disappeared.

The beneficial effects of the nutrients and limestone were probably due to several factors. The addition of the nutrients gave rise to more vigorous plants which are probably better able to withstand toxic agencies than the less vigorous plants. The nutrients undoubtedly also gave rise to more active bacterial life, which would hasten the decomposition and elimination of the vanillin and its decomposition products. The limestone undoubtedly had a similar effect on the bacterial activity. On oxidation, vanillin forms vanillic acid. If limestone is present to neutralize this acidity, harmful effects of this acid are prevented, and further decompo-

sition is favored. Limestone is thus especially effective in lessening the toxicity of vanillin on poor acid soils. The nutrients and limestone may also have increased the oxidizing power of the roots (28) and thus lessened the toxicity.

Experiment X.—This experiment was identical with Experiment IX, except that 4600 gm. per pot of Wabash silt loam soil were substituted for the Plainfield sand. Although the silt loam was decidedly acid, it was well supplied with phosphorus potassium and nitrogen. The plants were harvested after they had grown for 42 days. The results are shown in Table V.

TABLE V

EFFECTS OF VANILLIN ON THE GROWTH OF WHEAT ON A FERTILE SILT LOAM

Treatment	Dry Weight of Tops in gm.			
	Number of Pot	Series I Unlimed	Number of Pot	Series II Limed
Blank	17	3.594	25	2.923
Blank	18	3.491	26	2.878
Vanillin	19	4.131	27	3.330
Vanillin	20	3.431	28	2.996
Nutrients	21	4.210	29	3.665
Nutrients	22	4.072	30	3.681
Nutrients and Vanillin.	23	4.070	31	3.694
Nutrients and Vanillin.	24	3.915	32	3.596

In this comparatively fertile soil, as indicated in Table V, the vanillin had no apparent toxic effect. In practically every case, the vanillin-treated pots had just as good, if not a better growth than their corresponding controls. The calcium carbonate, which had been applied to the soil at the rate of nearly 10 tons per acre just the day before planting the seeds, had a depressing effect. In pot tests this is often the case with the first crop. This experiment indicates, as did previous ones, that vanillin is not a factor of any great importance in a soil having an adequate supply of fertility elements. It is reasonable to believe that when a plant has an inadequate food supply it cannot as effectively resist the poisonous effects of toxic substances, as when the food supply is adequate. In this connection it is also important to bear in mind that when the chemical and physical factors of soil fertility are supplied, the biological life is usually very active, which condition favors the decomposition and destruction of organic plant toxins. In fertile soils, the temporary combinations of toxins with soil constituents prevent injurious concentrations of toxins in the soil solution at periods when considerable amounts of toxins may be formed in the soil or carried into it. As these critical periods pass over, the toxins by leaching and decomposition gradually pass away.

SUMMARY

The work carried on by many investigators in the past shows that finely divided material has a marked inhibitory action on the toxicity of

many solutions to plants. This beneficial effect has generally been ascribed to the physical phenomenon of adsorption. In the case of quartz sand and finely powdered quartz particles, adsorption undoubtedly offers the true explanation. In the case of soils, there are large surface exposures and adsorption without question may play a large part in inhibiting the action of plant toxins. However, the great complexity of soil constituents suggests the possibility that plant toxins may combine chemically with certain soil constituents and thus be removed at least partly from the soil solution, resulting in a greatly lessened toxic action on plants.

The experiments reported in this paper indicate that chemical reactions are probably very important factors in lessening the harmful effects of plant toxins in soils. Calcium carbonate, a very common soil constituent, inhibited to a remarkable degree the toxicity, to wheat seedlings, of copper sulfate and copper nitrate. This result, while inexplicable as an adsorptive phenomenon, is readily explained on the basis of a chemical reaction between the copper salt and the calcium carbonate. The toxic effects of the strong base guanidine were markedly inhibited by the presence of either kaolin or an acid clay soil. The theory (44) of the acid nature of kaolin and of the acid silicates in acid upland soils offers a plausible explanation of these results. The acid kaolin apparently enters into chemical reaction with the strong base guanidine and removes it, at least partially, from solution.

The results of the present pot experiments with natural soils indicate (as do the results of other investigators) that vanillin can, at most, be but a very slight factor in soil fertility if the soil has an adequate supply of the fertility elements and is not acid in reaction. Here, again, calcium carbonate seems to inhibit or prevent the harmful effects of the toxic agent.

It is fully recognized that much more investigation along this line must be carried on before definite and general conclusions may be safely drawn. *However, it is believed that the data presented in this report show that in the amelioration of toxicity in soils, chemical reactions probably play fully as important a rôle as physical phenomena such as adsorption, and possibly the former have the greater effect.* In this connection the following statement (43) was made by one of the writers in a previous publication:

"The root hairs of plants are probably among the most delicate of all external organs in either plant or animal life. In the soil there are a great variety of processes going on, resulting undoubtedly in the formation not only of beneficial substances, but also of some harmful ones. If this is the case, it is probable that nature has made some provision for inhibiting the deleterious action of the harmful substances on the delicate root hairs. It seems possible that the silicates may form temporary combinations with these substances and thus prevent unfavorable action on the root hairs."

In the decomposition and disintegration of organic and mineral matter, acidic, basic and neutral substances are formed. In order for any of these substances to be toxic they must be appreciably soluble, or in other words attain a toxic concentration in the soil solution. The toxic substances in the soil solution strive at all times to attain a condition of equilibrium, chemically and physically, with the solid soil constituents, and they may combine directly or react by double decomposition with these constituents, if the equilibrium concentration is disturbed. If the equilibrium concentration is near or lower than the toxic concentration, then harmful effects on plants are prevented. This seems to be the condition that usually exists in fertile soils. By leaching or further decomposition the toxins are then gradually removed or destroyed. The chemical and physical constitution of most agricultural soils seems to be such that, with proper tillage and the use of lime when needed the injurious action of toxins which may be present or may arise in various ways, is entirely or to a large degree prevented.

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PLATE I

Fig. 1.—Unlimed cultures. The toxicity of copper sulfate to wheat seedlings in quartz sand cultures. Concentration of copper sulfate left to right: control, N/300, N/200, N/100, N/50, and N/10.

Fig. 2.—Limed cultures. The effect of calcium carbonate in inhibiting the toxicity of copper sulfate to wheat seedlings in quartz sand cultures. Concentrations of copper sulfate left to right same as in figure 1.

Fig. 3.—The toxicity of vanillin to wheat in Plainfield sand cultures, and the effects of limestone and fertilizers in inhibiting this toxicity.

No. 1—Control.
No. 3—Vanillin.
No. 5—Fertilizers.
No. 7—Fertilizers and vanillin.
No. 9—Lime.
No. 11—Lime and vanillin.
No. 13—Lime and fertilizers.
No. 15—Lime and fertilizers and vanillin.

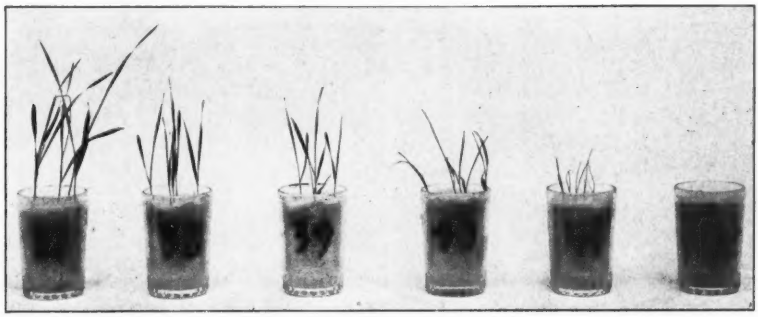

Fig. 1

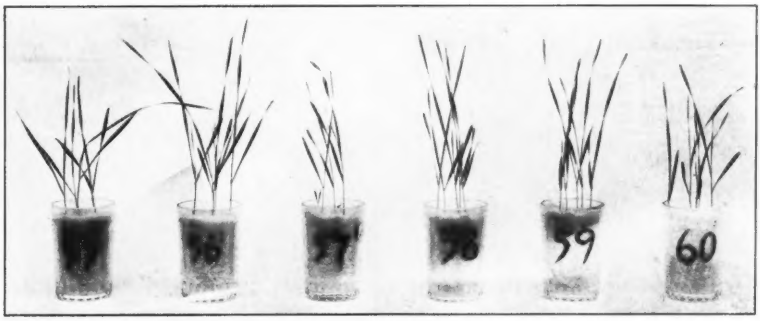

Fig. 2

Fig. 3

Fig. 1

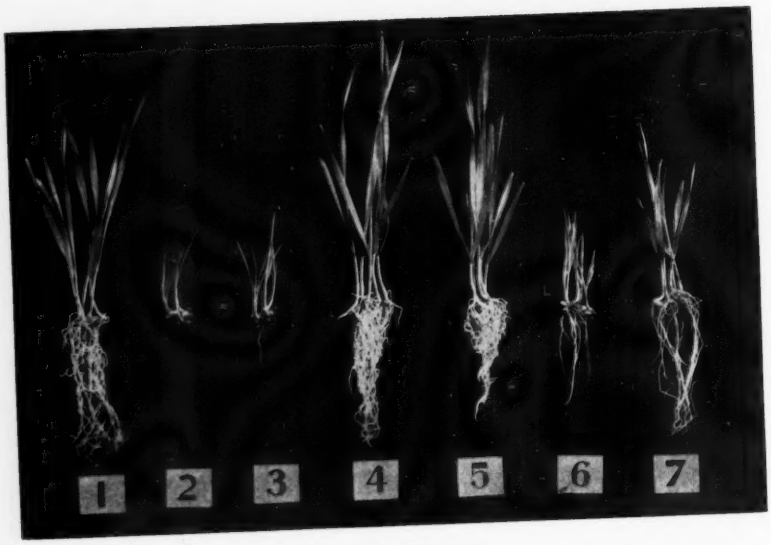

Fig. 2

PLATE II

Fig. 1.—The effect of calcium carbonate in inhibiting the toxicity of copper nitrate to wheat in quartz sand cultures with various additions indicated below. Concentration of copper nitrate used was N/100.

No. 18—Quartz sand, control.

No. 19—Quartz sand and copper nitrate.

No. 20—Quartz sand, copper nitrate and calcium carbonate.

No. 21—Quartz sand, copper nitrate and quartz flour.

No. 22—Quartz sand, copper nitrate, quartz flour and calcium carbonate.

No. 23—Quartz sand, copper nitrate and kaolin.

No. 24—Quartz sand, copper nitrate, kaolin and calcium carbonate.

Fig. 2.—The toxicity of sodium arsenite to wheat in quartz sand cultures with various additions indicated below. Concentration of sodium arsenite used was N/800.

No. 1—Quartz sand, control.

No. 2—Quartz sand and sodium arsenite.

No. 3—Quartz sand, sodium arsenite and calcium carbonate.

No. 4—Quartz sand, sodium arsenite and quartz flour.

No. 5—Quartz sand, sodium arsenite, quartz flour and calcium carbonate.

No. 6—Quartz sand, sodium arsenite and kaolin.

No. 7—Quartz sand, sodium arsenite, kaolin and calcium carbonate.

PLATE III

Fig. 1.—The toxicity of guanidine carbonate to wheat in quartz sand cultures. Concentrations of guanidine carbonate used left to right were: control, 25, 50, 200, 500 and 1000 parts per million.

Fig. 2.—The effect of kaolin in inhibiting the toxicity of guanidine carbonate to wheat in quartz sand cultures. Concentrations of guanidine carbonate used, left to right same as in figure 1. Cultures identical with figure 1, except that 10 per cent of kaolin was used. A comparison of figures 1 and 2 indicates a remarkable beneficial effect on root growth of the kaolin, even with the controls.

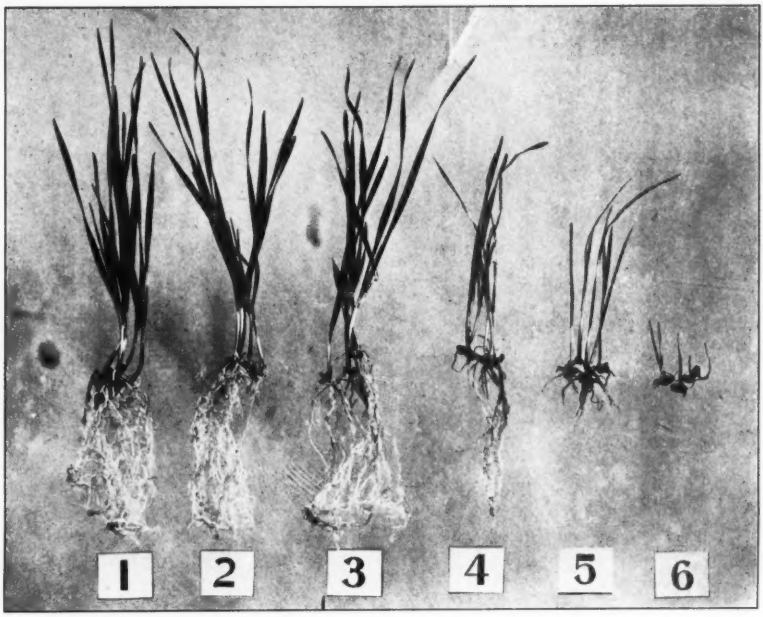

Fig. 1

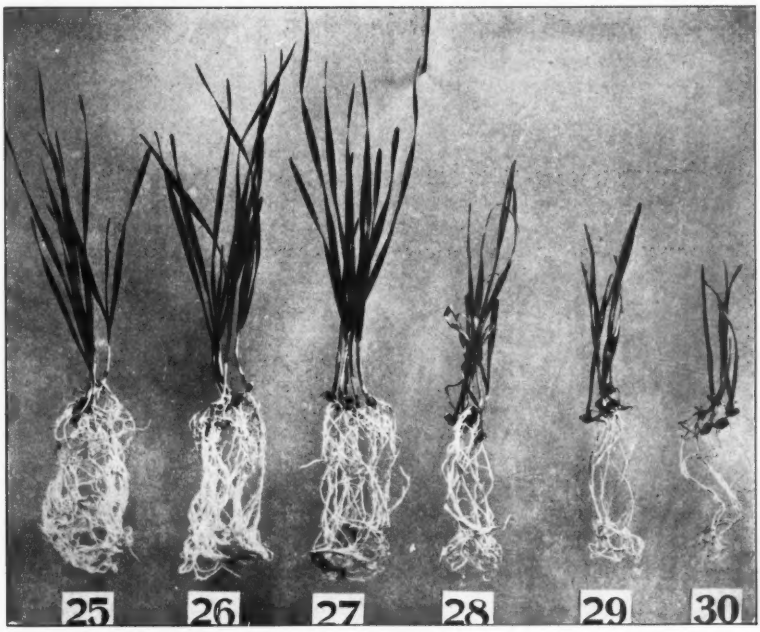

Fig. 2

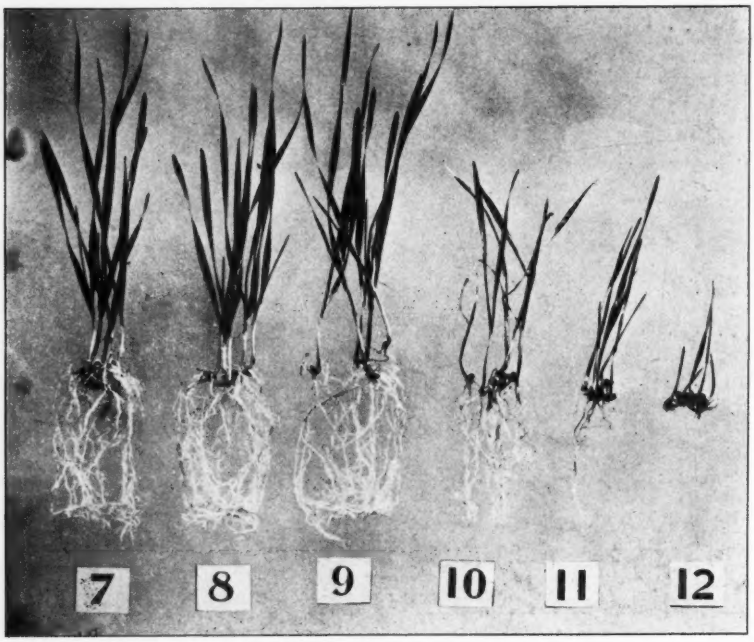

Fig. 1

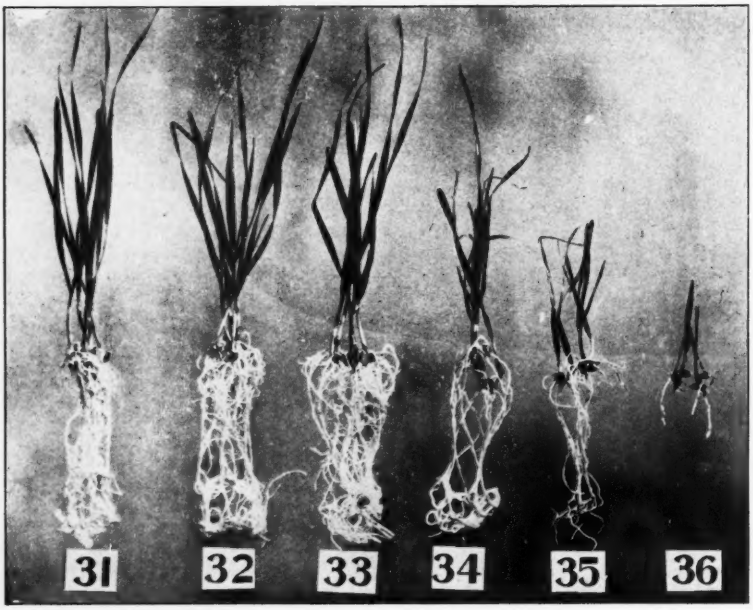

Fig. 2

PLATE IV

The effect of calcium carbonate on the toxicity of guanidine carbonate to wheat. Concentrations of guanidine carbonate used, left to right, were as follows: control, 25, 50, 200, 500 and 1000 parts per million.

Fig. 1.—Quartz sand cultures, with calcium carbonate. Identical with figure 1 (Plate III), except that calcium carbonate was added.

Fig. 2.—Quartz sand cultures with kaolin and calcium carbonate. Identical with figure 2 (Pl. III), except that calcium carbonate was added.

PLATE V

The effect of various substances in inhibiting the toxicity of guanidine carbonate to wheat.

Fig. 1.—Concentration of guanidine carbonate used, 1000 parts per million. Other treatments as follows:

No. 6—Quartz sand.
No. 12—Quartz sand and calcium carbonate.
No. 18—Quartz sand and quartz flour.
No. 24—Quartz sand, quartz flour and calcium carbonate.
No. 30—Quartz sand and kaolin.
No. 36—Quartz sand, kaolin and calcium carbonate.

Fig. 2.—Concentration of guanidine carbonate used 500 parts per million, excepting No. 8, which received none. Other treatments as follows:

No. 8—Quartz sand, control.
No. 9—Quartz sand and toxin.
No. 10—Quartz sand, toxin and calcium carbonate.
No. 11—Quartz sand, toxin and quartz flour.
No. 12—Quartz sand, toxin, quartz flour and calcium carbonate.
No. 13—Quartz sand, toxin and acid clay.
No. 14—Quartz sand, toxin, acid clay and calcium carbonate.

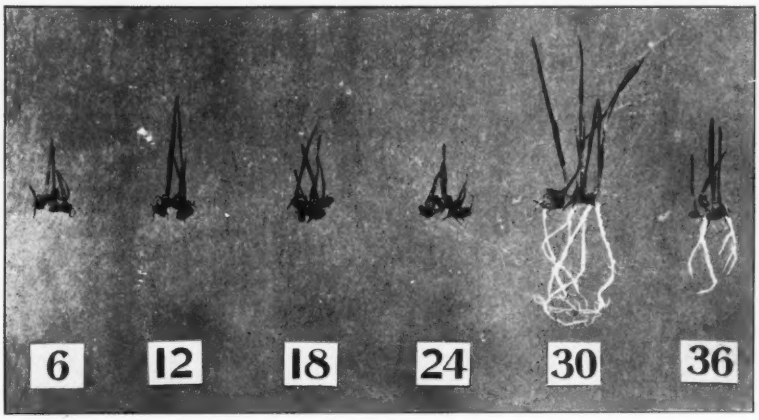

Fig. 1

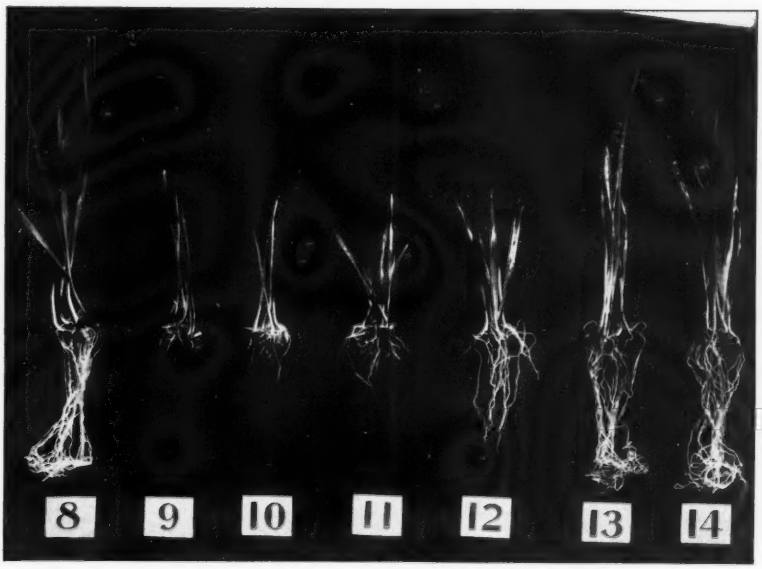

Fig. 2

THE EXTRACTION AND SATURATION OF SOILS WITH VOLATILE ANTISEPTICS[1]

By

J. P. du Buisson

Introduction

That partial sterilization of soils is a factor to be considered in soil fertility has been demonstrated beyond doubt during the last thirty years. Partial sterilization may be effected either by heating the soil or by treating it with a volatile, a non-volatile, or a solid antiseptic. However, heating soil and treating it with volatile antiseptics are the two methods that have especially been studied by investigators up to the present time. The effect of heat on soil was first noticed by early bacteriologists. Since then, this phenomenon has been studied variously and extensively. Many conflicting theories have been offered as a solution for the cause of these beneficial effects. Some have attributed them to a biological, others to a chemical or mechanical, change in the soil itself, and still others to all three factors combined.

The present report is limited to the study of volatile antiseptics only. The object of this investigation was to determine, if possible, whether there is any essential difference in the effect of saturation as compared with extraction of different soil types with volatile antiseptics. With this in view, the effect of both saturation and extraction, on separate samples of the same soil types, was studied, in so far as these substances influenced plant growth, ammonification, nitrification, and the total water-soluble salts in the soils used in the experiment.

A comparison of the effects of saturation and extraction was made for the purpose of testing the theory, advanced by Greig-Smith (17), that the volatile antiseptics dissolve the "agricere" that covers the surfaces of the soil particles and by so doing enables higher plants and also bacteria to obtain more nutriment. Extraction with the antiseptics should remove the "agricere" more completely than mere saturation, and might, therefore, be expected to produce a condition more favorable to both higher plants and bacteria.

[1] A thesis submitted, to the faculty of the Graduate School of Cornell University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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I. Historical

1. *Increase of Productivity due to Treatment of Soil with Volatile Antiseptics*

The first record of an antiseptic treatment of soil seems to be that of Oberlin (37). After using carbon bisulfide as an insecticide in some of his vineyards that were attacked by *Phylloxera,* he noted a marked increase in the growth of the vines. Girard (15) found beneficial results from this antiseptic with sugar beet. Pagnoul (38) observed the same phenomenon with buckwheat and with mustard. His observations with mustard are corroborated by Wagner (51). Mach (32) reported greater yields with beets, oats, and potatoes, after the application of 200 gm. of carbon bisulfide to a square meter of soil. Wollny (53) obtained increased results in pot experiments as a result of carbon bisulfide treatment. Koch (23) found increased growth with buckwheat and mustard and beneficial results in vineyards. In 1911 the same author (24) reported the following relative green weights for buckwheat: untreated soil, 100; application of 200 c.c. ether, 153; of 500 c.c., 179. Hiltner and Störmer (20) obtained increased yields for buckwheat on soil treated with carbon bisulfide. Moritz and Scherpe (35) report an increased yield with potatoes and rye with carbon bisulfide. Nobbe and Richter (36), treating soil with ether, chloroform, carbon bisulfide and benzene, report the following relative dry weights: 118, 114, 122, 122, respectively, against 100 for untreated. Egorov (10) noticed higher yields of oats with carbon bisulfide treatment. Darbishire and Russell (9) found an increase in the yield of buckwheat and mustard on soil treated with chloroform, carbon bisulfide, ether and toluene. Likewise there was an increased yield of turnips on soil with formalin as an antiseptic. Russell and Hutchinson (43) obtained greater yields with wheat and rye on toluene-treated soil than on mustard soil. Sherpe (45) reports an increased yield of rye on soil treated with carbon bisulfide. Emmerrich, Leiningen and Loew (11) noticed a beneficial effect with carbon bisulfide on cane seedlings. Stone (48) similarly obtained better growth with lettuce after treating the soil with carbon bisulfide. Gainey (14), while experimenting with toluol, carbon bisulfide and chloroform, found increased yields with oats, wheat, and buckwheat.

It is evident from these results that the ability of partially sterilized soils to yield larger crops is a general one. Furthermore, it holds for all the soils studied and for the various volatile antiseptics examined. According to Russell and Hutchinson (43) this seems to be true for all plants except for those of the leguminous order.

2. *The Effect of Partial Sterilization on the Ammonifying and Nitrifying Power of the Soil*

Here will be considered some of the investigators who noted the

effect of volatile antiseptic on the nitrifying and ammonifying power of the soil. The data are very conflicting on this point. Wagner (51) found a decrease in nitrification with carbon bisulfide treatment. Coleman (8) reported an immediate inhibiting effect on nitrate formation on soil treated with carbon bisulfide, but after a time found an increase.

Lipman (27), on the other hand, reports a beneficial effect both for ammonification and nitrification. Störmer (49) and Scherpe (45) both observed an increase in ammonification but a detrimental effect on nitrification. The results of these two investigators are corroborated by the more extensive work done in England, especially by Russell and Hutchinson (43), who found the production of ammonia after partial sterilization at first slow, then rapid, and then more or less constant. Goodey (16), Hutchinson and MacLennan (21), Buddin (5) and others report similar results.

Laidlaw and Price (26) noted that in partially sterilized soil more ammonia was produced but a cessation of the nitrifying process took place. On the other hand, Chaudon de Briailles (7), Pagnoul (38), Koch (24) and Fred (13) all report that partial sterilization enhanced the process of nitrification after a considerable duration of time. Notwithstanding the fact that the reported results are somewhat conflicting, there appears to be a pronounced indication that the volatile antiseptic treatment of the soil inhibits, at least for a time after the treatment, the nitrification process. This inhibition is then followed by a marked stimulation.

3. *The Effect of Volatile Antiseptics upon the Total Water-Soluble Salts in the Soil*

The literature on this subject is very meager. Most of the investigators have studied the availability of plant-food due to the effect of partial sterilization with heat. Koch (23) does not believe that carbon bisulfide directly liberates plant nutrient elements, but states that as carbon bisulfiide stimulates plant growth, it is reasonable to expect that more plant-food is removed from the soil. Darbishire and Russell (9) found that soil treated with carbon bisulfide was able to supply the plants with 75 per cent more phosphoric acid, 40 per cent more potash and 50 per cent more nitrogen than the untreated soil. Using plants as indicators, Russell and Hutchinson (43) showed that more nitrogen, phosphorus and potassium were removed from soil treated with carbon bisulfide than from the untreated soil.

The partial sterilization of the soil by heat has been more extensively studied. Frank (12), Kruger and Schneidewind (25), Lyon and Bizzell (31), Pickering (40), and Stone (48) demonstrated that soluble plant and bacterial nutrients have been increased in partially sterilized soil with such treatment. As partial sterilization with volatile antiseptics is

analogous in many respects to sterilization with heat, it would seem that more plant nutrients should become available when the soil is treated than when untreated with such volatile antiseptics.

4. *Methods of Treating the Soil with Volatile Antiseptics and the Effect upon Biological Processes*

Various methods have been employed for treating the soil with volatile antiseptics. The general method under field conditions seems to have been the introduction of the antiseptic by means of holes bored in the soil at regular intervals. Mach (32) added 200 gm. of carbon bisulfide per square meter. Koch (23) applied 60 c.c. of carbon bisulfide and ether respectively, to 20 kg. of soil. Hiltner and Störmer (20) applied 516 gm. of carbon bisulfide to a square meter of soil as follows: three holes were made, each 30 cm. deep, in every square meter of soil; the carbon bisulfide was poured into the holes, which were immediately filled up with soil; the plot of soil was afterward spaded over to insure equal distribution of the antiseptic; the soil was not seeded nor samples taken for biological determination, as the case might be, until all odor from the antiseptic had disappeared. Koch (24) and Fred (13) used similar methods for the different antiseptics, the latter working with pot experiments.

Nobbe and Richter (36) applied to the first set of experiments 62 gm. of ether to each pot of 3600 gm. of soil. To the second set having the same quantity of soil and known as the ether emulsion treatment, 300 c.c. of ether and 300 c.c. of water were added. The soils were then thoroughly mixed with the antiseptic, put into air-tight boxes with a small receptacle containing ether, for a given time, and then exposed to the free atmosphere. In a third test, hydrogen peroxide was used. A 30 per cent solution diluted with 750 c.c. of water was applied to each pot of soil as above stated. The soil was thoroughly mixed and was then considered ready for the growth of plants. The authors also report experiments with ether, carbon bisulfide, chloroform and benzene where the method of treatment was identical as under the second set described above.

Darbishire and Russell (9) added 25 c.c., respectively, of carbon bisulfide, chloroform, toluene, ether and benzene to 1 kg. of soil. The pots with soil were covered and allowed to stand for a period of one week. The soil was then spread open in a thin layer and the antiseptics allowed to evaporate. The evaporation took about 3 days. Russell and Hutchinson (43) employed a similar method but used only 2 c.c. of toluene per kilogram of soil where plants were to be grown. For the determination of the ammonifying and nitrifying power of the soil, 40 gm. of toluene were added to a receptacle holding 800 gm. of soil. A second series was treated similarly, but the toluene allowed to evaporate at the end of 3 days, while in the first series the antiseptic remained in the soil during the whole period of experimentation.

5. *The Relative Effect on Crop Yields of the Different Volatile Antiseptics in the Partial Sterilization of Soils*

Though several investigators have studied different antiseptics, carbon bisulfide has been used to a greater extent than any other. Koch (23) reports that better crop yields were obtained with carbon bisulfide as an antiseptic than with ether under similar conditions. In 1911 the same author (24) pointed out an increase of crop yield with increase of quantities of carbon bisulfide and ether used, but not necessarily proportional to the amount applied. The effectiveness again was in favor of carbon bisulfide.

According to the crop yields reported by Nobbe and Richter (36), the order of effectiveness of the antiseptics used was as follows: carbon bisulfide, benzene, chloroform, and ether. It would seem from the relative weights reported for buckwheat by Darbishire and Russell (9) that carbon bisulfide is better than chloroform and the latter superior to ether as an antiseptic. For mustard the following order was observed by the same authors: chloroform, benzene, carbon bisulfide, and toluene, with an average relative yield of 118 against 100 for the untreated soil. From the meager literature available it is apparent that some antiseptics are more effective than others.

6. *Suggested Theories in Explanation of the Effect of the Partial Sterilization on Soil Fertility*

A number of views have been advanced to explain the cause of the beneficial effect that higher plants and bacterial flora derive from partial sterilization of soils by volatile antiseptics. These will be discussed in the order of their priority.

Koch (23) holds that partial sterilization has a direct stimulating effect upon the higher and lower forms of plant life in the soil. He is not the only investigator to produce data substantiating this veiw. Nobbe and Richter (36), Egorov (10), Fred (13) and others are of the same opinion.

Hiltner and Störmer (20) advanced the so-called indirect selective theory of the antiseptics as related to the growth and activity of bacterial flora. They maintain that the harmful organisms are suppressed, whereas the beneficial bacteria are stimulated by the changes brought about in the soil as a result of such treatment.

In 1909 Russell and Hutchinson (43) announced their protozoa theory. These investigators believe that the protozoa in the soil hold in check the multiplication of the bacteria, especially those of the ammonifying type. They hold that an antiseptic destroys most of the large organisms that prey on the bacteria. The latter, although temporarily suppressed by the antiseptic, are later able to multiply unhindered and so attain numbers greatly in excess of those found in normal soils. The

greater number of bacteria is assumed to cause more plant nutrients and nutrient elements to be elaborated and, consequently, larger crop yields are produced. Fred (13), Sherman (47) and others, however, have given data showing that some protozoa are not so detrimental to bacteria as Russell and Hutchinson believe.

Previous to Russell and Hutchinson's work, the existence of protozoa in soil was reported by Celli and Fiocca (6), who isolated 6 species of amœbæ. Other investigators in England who have reported the presence of protozoa in the soil are Goodey (16), Martin (33), and Martin and Lewin (34). In Germany, Hiltner (19), Tsujitani (50), Emmerich, Leiningen and Loew (11), Killer (22) and others have noted the same phenomenon. In the United States the presence of such organisms has been observed by Gainey (14), Lodge and Smith (28), Rahn (41), and Sherman (46, 47). Peck (39) has observed their presence in Hawaii soil. Loew (29, 30) reported their presence in the soil of the Alps, in Japan, the Island of Borkum and in Porto Rico.

Bolley (1, 2, 3) considers the parasitic fungi in the soil as the chief cause, in many cases, of poor vegetative growth. The effect of the partial sterilization is to destroy or check the parasitic fungi and, consequently, allow the plant to grow unhampered.

Greig-Smith (17) has advanced the "agricere" theory. The agricere is considered to be a waxy substance, which covers, as it were, the soil particles. When the soil is treated with antiseptics this agricere is destroyed and conditions are rendered more favorable for the liberation of nutrients for plants and bacteria.

Greig-Smith (17) and Bottomley (4) have proved the presence of bacterio-toxins in soil. Greig-Smith found them to a greater degree in poor than in rich soil. He claims that the toxins check bacterial activity in the soil.

Summary of Literature

1. Treatment of soil with volatile antiseptics has a definite beneficial effect on plants subsequently grown on such soil.

2. The ultimate effect of partial sterilization is an increased production of ammonia and nitrates.

3. Heat sterilization of soil liberates plant nutrients. As the treatment of soil with volatile antiseptics is somewhat similar in effect to heat sterilization, a like phenomenon may probably result.

4. In general, the application of volatile antiseptics to field soil is made in holes bored for that purpose. In greenhouse and laboratory experiments the antiseptic either is allowed to volatilize after treatment or is left in the soil.

5. Some volatile antiseptics seem to be more effective sterilizing agents than others.

6. Different theories as to the beneficial effects of volatile antiseptics may be stated as follows:

a. Koch believes that antiseptics have a direct stimulating effect on plant and bacterial life.

b. Hiltner and Störmer consider the action as a disturbance of the equilibrium of the soil flora.

c. Russell and Hutchinson attribute the beneficial effects of volatile antiseptics to the suppression of the soil protozoa, which are considered to hamper ammonification.

d. Bolley considers the checking of certain harmful parasitic fungi in many cases as the real influence of partial sterilization.

e. Greig-Smith believes that the solution of certain waxy material in the soil by volatile antiseptics affords conditions for a more ready availability of plant nutrients.

II. Conditions and Methods of Experimental Procedure

The experimental data embodied in this report were derived from two sources: greenhouse studies and laboratory investigations.

Both parts of the report were carried out in the Soil Technology Department of Cornell University, beginning with the spring of 1915 and continuing through the summer of 1916.

1. *Pots.*

Four classes of receptacles were used for the vegetative part of the experiment.

(a) Glazed crockery pots of ½-gallon capacity, 4½ inches in diameter and 6 inches deep, holding 2½ kg. of dry soil.

(b) Small ordinary unglazed flower pots of 500-gm. capacity, 5 inches in diameter and 5 inches deep. These were used for the ether-treated soils described under Experiment I.

(c) Unglazed clay flower pots of ½-gallon capacity, 6 inches in diameter and 6¼ inches deep. These pots were used in Experiment XI for the growth of the wheat crop, having been previously dipped into paraffin in order to cut down evaporation and diffusion through the sides.

(d) Glazed crockery pots of 2-gallon capacity, 8 inches in diameter and 6 inches deep, each holding about 5 kg. of soil.

It was noticed that the root systems which developed in the smaller pots were too crowded, and to eliminate this unfavorable condition larger pots were employed, notwithstanding the fact that greater quantities of the volatile solvents were necessary.

2. *Soils.*

Dunkirk clay loam and Volusia silt loam soils were used. Both are typical soils in the vicinity of Cornell University. The Dunkirk clay loam was surface soil obtained from the experimental plots of Caldwell Field. The Volusia silt loam was surface soil from the Stevens farm on Turkey Hill.

Unpublished results of bulk analysis for Dunkirk clay loam soil are as follows:

FROM 9 SAMPLES OF TOMPKINS COUNTY SOIL

	Surface %	Subsoil %
C (organic carbon)	1.670	0.440
CO_2	trace	0.260
K_2O	1.740	2.110
CaO	0.430	0.830
MgO	0.450	0.690
Na_2O	1.090	1.280
N	0.186	0.082
P_2O_5	0.123	0.126

Unpublished results of bulk analysis for Volusia silt loam soil are as follows:

FROM 11 SAMPLES OF TOMPKINS COUNTY SOIL

	Surface %	Subsoil %
C (organic carbon)	1.960	0.650
CO_2	trace	trace
K_2O	1.630	1.970
CaO	0.270	0.240
MgO	0.240	0.250
Na_2O	0.850	0.960
N	0.169	0.086
P_2O_5	0.153	0.127

The soil in a majority of cases was obtained in bulk. After being allowed to dry, it was reworked in order to get rid of lumps and stones, the latter being especially numerous in the Volusia silt loam. The soil was then sieved through a 2-mm. sieve, except that used in Experiment I, which was passed through a 10-mm. sieve.

3. *Treatment of Soils.*

In order that the discussion of the different experiments may be easier to follow, and to avoid repetition, a description will be given of the various solvents employed with an explanation of the different terms used.

The organic solvents utilized in this experimentation were alcohol, benzene, ether, commercial gasoline, and toluene.

It will be seen from the experiments which follow that the alcohol treatment was applied to both types of soil on three different occasions. Ether, gasoline, and toluene, on the other hand, were each applied twice to Dunkirk clay loam soil as a treatment and only once to Volusia silt loam. Benzene was applied only in Experiment I to Dunkirk clay loam.

The treatment in general consisted in extracting and saturating each type of soil with the individual solvents.

(a) Extracted soil.

By extraction it is to be understood that definite quantities of the solvent were applied to definite quantities of soil. The ratio was a variable one. In case of alcohol and ether, for example, the ratio was 3

of solvent to 1 of the soil. With gasoline, benzene, and toluene the ratio was 4 to 1. The previously prepared soil was placed in a receptacle and the proper amount of solvent added. The soil was thoroughly stirred three times a day as long as the extraction lasted, which varied from 1 day for alcohol, to 8 days for gasoline. At the end of the extraction the solvent was siphoned off. The soil thus treated was then spread out on thick paper in a well ventilated dark room until, as far as could be determined by the odor, the solvent had disappeared entirely. The time varied also in this case from 3 days for alcohol to 10 days for gasoline. Moisture determinations were then made and the soil was ready for experimentation.

(b) Saturated soil.

Each soil was saturated with the respective solvents during the same period as in the extraction above described. The soil was then spread out and the solvent allowed to evaporate. The drying continued for the same length of time as for the extracted soil. Moisture determinations were then made and the soil was considered ready for use.

(c) Untreated soil.

The soils spoken of as untreated in this report are the respective soils, which were worked up in the same manner as the portions taken for the two treatments just described. The soil was spread out for the same period as those described under the treatments above, and after moisture determinations were made were ready for use.

4. *Moisture Control.*

The soil used for the vegetative work in the greenhouse was kept at 30 per cent moisture. The incubated soil was kept at 25 per cent moisture. Both calculations were on the moisture-free basis. The former percentage was considered to be the optimum moisture for plant growth. The 25 per cent moisture was used for all the incubated soils on which tests were run for nitrification, ammonification and total water-soluble salts. This percentage was considered the optimum moisture content for organisms.

5. *Incubation.*

To test the ammonifying and nitrifying power of the soil, incubation tests were run. These were carried out by placing 100 gm. of air-dried soil in 8-ounce bottles plugged with cotton. Incubation temperature was that of the laboratory.

6. *Methods Used for Determining Nitrates, Ammonia and Total Soluble Salts.*

The methods used for determining nitrates and ammonia were those described in Bulletin 31 of the Bureau of Soils of the United States Department of Agriculture.

7. *General Plan of Experimentation.*

That the data may be presented in as clear a form as possible, a general plan will be presented first and discussed in detail afterwards. The

vegetative and laboratory parts of the work were divided up into the following separate experiments:

Vegetative Experiments

Experiment I. Oats, spring of 1915 (followed by buckwheat of Experiment IV, summer of 1915).

Experiment II. Wheat, summer of 1915 (followed by oats of Experiment V, 1915-1916).

8. *Diagramatic Plan of Experimentation*[2]

Experiment I Direct effect of volatile antiseptic on *oats.*	*Experiment II* Direct effect of volatile antiseptic on *wheat.*	*Experiment III* Direct effect of volatile antiseptic on *oats.*
Experiment IV Residual effect of volatile antiseptic on *buckwheat* after *oats* (Experiment I).	*Experiment V* Residual effect of volatile antiseptic on *oats* after *wheat* (Experiment II).	*Experiment VI* Effect of water-soluble alcoholic extract from soil treated with volatile antiseptic as in Experiment III upon *oats* in water cultures.
	Experiment VII Direct effect of volatile antiseptic on the chemical condition of the soil treated with volatile antiseptic as in Experiment II.	*Experiment VIII* Direct effect of volatile antiseptic on the chemical condition of the soil treated with volatile antiseptic as in Experiment III.
Experiment IX Residual effect of volatile antiseptic upon the chemical condition of the soil after *oats* (of Experiment I) and *buckwheat* (Experiment IV).	*Experiment X* Residual effect of volatile antiseptic upon the chemical condition of the soil after *wheat* (Experiment II) and *oats* (Experiment V).	*Experiment XI* Physical condition of the soil upon certain chemical factors. Soil of Experiment III studied directly after the *oats.*

Experiment XII

The direct effect of volatile antiseptics upon development of acid in the soil. Soil treated exactly as in Experiments I, II and III.

Experiment III. Oats, spring and summer, 1916. (Soil later studied chemically. See Experiment XI.)

Experiment IV. Buckwheat following oats. (See Experiment I.)

Experiment V. Oats following wheat. (See Experiment II.)

Experiment VI. Water culture experiments.

Laboratory Investigations

Experiment VII. Soil incubated for 3, 6 and 12 weeks. The soil was treated with volatile antiseptics exactly as in Experiment II.

[2] It is to be noted that Experiments I, II and III are comparable, also Experiments IV and V, Experiments VII and VIII, and Experiments IX and X. Experiments VI, XI and XII are each to be considered separately.

Experiment VIII. Soil incubated for 2, 4 and 6 months. The soil received the same treatments with volatile antiseptics as in Experiment III.

Experiment IX. Soil incubated and studied chemically after harvesting the buckwheat of Experiment IV. The buckwheat followed an oats crop (Experiment I).

Experiment X. Soil studied chemically directly from pots after harvesting crop of oats (Experiment V) which followed a crop of wheat (Experiment II).

Experiment XI. Soil studied chemically from pots and simultaneously incubated after harvesting a crop of oats (Experiment III).

Experiment XII. The study of the development of acids in soils immediately after the treatment with alcohol as an antiseptic.

III. Vegetative Experiments

As outlined in the general plan, the experimental part is subdivided into different experiments which will be discussed in their numerical order.

Experiment I

The Effect of Volatile Antiseptics Applied to the Soil upon the Following Oat Crop

Both Volusia silt loam and Dunkirk clay loam soils were extracted and saturated with 90 per cent alcohol in this experiment. Furthermore, benzene, ether, toluene and gasoline, respectively, were applied to Dunkirk clay loam alone. After the soil was treated as already described, 2 kg. of soil were weighed out in duplicate, into the ½-gallon glazed crockery pots. In case of the ether-treated soils, however, only 500 gm. of soil were used in the small flower pots.

The soils stood in the pots for 8 days before seeding. On April 13, 1915, all the pots were seeded with oats, 16 seeds to a pot. When the plants were 2 inches high, all were removed except eight of uniform size. At this time, 300 gm. of clean quartz sand were spread over the surface of the soil in each pot. This was to act as a mulch and decrease the amount of water lost by evaporation.

The seeds in the treated soil in most cases germinated a day, and in the case of gasoline from 2 to 3 days, later than the seeds in the untreated soil. This, however, was not the case with the seeds in the ether-treated soil, the germination period being the same as that of the seeds in the untreated soil.

During the first 4 to 6 weeks of growth, the plants in all the pots with treated soil (ether being an exception again) did not show the same amount of growth as the plants in the untreated soil. The saturated soil maintained a slight advantage over the extracted soil. From this period on, however, there was a marked increase in growth of the plants on the treated soils, as can be seen from the dry weight of the plants (Table I),

which were harvested on July 21, 1915, after the crop had passed the blooming stage and was about to ripen.

Conclusions Regarding Experiment I

These results indicate a decided benefit to crop growth in favor of the treated soils. The saturated soils responded better to the treatment than extracted soils. This is shown not only by the average data but also by every individual treatment.

The 90 per cent alcoholic antiseptic seems to have affected the crop growth most beneficially, ether next, then benzene, and finally, toluene. The gasoline antiseptic had a detrimental effect, the extracted treatment even more so than the saturated treatment.

TABLE I

RESULTS OBTAINED WITH OATS GROWN ON SOILS PREVIOUSLY TREATED WITH VOLATILE ANTISEPTICS, EXPRESSED AS AVERAGE DRY WEIGHTS

(Planted April 13, 1915; harvested July 21, 1915)

Soil Type	Volatile Antiseptic	Untreated		Saturated Soil		Extracted Soil	
		gm.	Relative Weights	gm.	Relative Weights	gm.	Relative Weights
VSL[1]	Alcohol	2.50	100	2.75	110	2.50	100
DCL[2]	Alcohol	1.70	100	3.15	185	2.90	170
DCL	Ether[3]	1.05	100	1.65	157	1.25	119
DCL	Gasoline	1.75	100	1.65	94	1.30	74
DCL	Toluene	1.95	100	2.35	121	2.20	113
DCL	Benzene	2.05	100	2.65	129	2.30	112
Grand Average		1.80	100	2.40	133	2.07	115

[1] Volusia silt loam.
[2] Dunkirk clay loam.
[3] With ether treatment only 6 plants were left in each pot to mature.

All of the antiseptics lengthened the period for germination except ether, which treatment produced no variation from the untreated soil.

Experiment II

The Effect of Volatile Antiseptics Applied to the Soil upon the Following Wheat Crop

This experiment was begun in August, 1915. The organic solvents used were alcohol, ether and toluene on both Dunkirk clay loam and Volusia silt loam.

Two-kg. flower pots were used into which were weighed 1800 gm. of alcohol and toluene-treated soil, respectively. In the case of the ether treatments, 1400 gm. of soil were used. On September 2, 1915, these pots were seeded with Galgalos wheat, 20 seeds to a pot. The young plants were later thinned to 10 plants of uniform size.

The first important consideration here was the germinating power of the seeds in these differently treated soils. Invariably, the seeds in the untreated soil germinated 2 or 3 days sooner than on the treated soil and showed for the first 3 weeks a distinctly better growth. This was es-

pecially noticed with the alcohol and the toluene treatments. The ether did not show this inhibiting effect. As the growing period progressed, the plants on the treated soils gradually improved, and showed a healthier color and a more vigorous growth at the end of 5 weeks.

On account of a bad attack of mildew, to which wheat is especially subject when grown in the greenhouse during the summer, this crop had to be harvested on October 17, thus growing for only 6 weeks. Although these results are not so reliable and conclusive as from plants grown to maturity, nevertheless the dry weights shown in Table II show a distinct tendency in favor of the treated soil.

TABLE II

RESULTS OBTAINED WITH WHEAT GROWN ON SOILS PREVIOUSLY TREATED WITH VOLATILE ANTISEPTICS, EXPRESSED AS AVERAGE GREEN AND DRY WEIGHTS

(Planted September 3, 1915; harvested October 17, 1915)

Soil Type	Volatile Antiseptic	Untreated Soil			Saturated Soil			Extracted Soil		
			Weight of Dry Matter			Weight of Dry Matter			Weight of Dry Matter	
		Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.
DCL	Alcohol	3.40	0.80	100	6.15	1.35	169	6.65	1.45	181
VSL	Alcohol	5.70	1.20	100	7.20	1.55	127	5.75	1.35	112
DCL	Ether	4.60	0.95	100	7.55	1.37	144	9.35	1.65	173
VSL	Ether	5.50	1.20	100	7.30	1.32	110	8.65	1.60	133
DCL	Toluene	4.60	0.95	100	5.85	1.25	131	5.35	1.20	126
VSL	Toluene	5.30	1.05	100	7.45	1.40	133	7.75	1.40	133
Grand Average		4.85	1.02	100	6.91	1.37	135.7	7.25	1.44	143

Conclusions Regarding Experiment II

The crop in this experiment also responded with greater yields on the treated soils than on the untreated ones. There is a general tendency in favor of the extracted treatments, although there are marked exceptions there, also. The order of effectiveness of the antiseptics according to the results are alcohol, ether and toluene, respectively. The Dunkirk clay loam in general responded better than Volusia silt loam, except for the ether treatment, where the reverse was true. Alcohol and toluene retarded germination, while ether seemed to have no influence, either stimulating or retarding.

Experiment III

The Effect of Volatile Antiseptics Applied to the Soil upon the Following Oat Crop

In the previous experiment the largest pots used were ½-gallon pots. The object in Experiment III was to study the various treatments on larger quantities of soil. Four kg. of soil were used in 2-gallon pots. The Dunkirk clay loam was stock soil which had been kept for 3 years

in the store-room. The Volusia silt loam was the same as previously described.

Both types of soil were extracted with 70 per cent alcohol. The gasoline treatments on both types of soil were in the ratio of 4 parts of gasoline to 1 part of soil. Five and 10 days were allowed, respectively, for the evaporation of the alcohol and gasoline.

The soils, after standing for 6 days in the pots, were seeded to oats on March 9, 1916. Twenty-five seeds were planted to a pot, but only fifteen of the resulting plants were allowed to grow. The same phenomenon was observed as in previous experiments, to wit, that the treatment had a retarding effect for the first few weeks of growth. After this period, the plants on the alcohol-treated soil began to show a more rapid growth, extending until the harvest time, June 1, 1916. The plants were in full bloom at harvesting.

TABLE III

RESULTS OBTAINED WITH OATS GROWN ON SOILS PREVIOUSLY TREATED WITH VOLATILE ANTISEPTICS, EXPRESSED AS AVERAGE GREEN AND DRY WEIGHTS

(Planted March 9, 1916; harvested June 1 and June 30, 1916)

Soil Type	Volatile Antiseptic	Untreated Soil			Saturated Soil			Extracted Soil		
			Weight of Dry Matter			Weight of Dry Matter			Weight of Dry Matter	
		Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.
DCL	Alcohol	91.90	16.75	100	107.45	21.20	120	133.35	26.10	155
VSL	Alcohol	61.25	10.50	100	100.40	19.70	187	91.75	17.90	170
DCL	Gasoline	32.50	8.95	100	35.75	8.85	98	31.00	7.05	79
VSL	Gasoline	72.05	22.55	[1]100	66.60	17.60	78	71.15	23.10	102
Grand Average		64.45	14.69	100	77.55	16.84	120.7	81.81	18.79	126.5

[1] It should be remembered that the crops from the alcohol-treated soils were harvested on June 1, 1916, whereas the gasoline-treated soil was not harvested until June 30. This is one factor that accounts for the difference in weight from the untreated soil of the Volusia silt loam. The kernels were riper and thus had very little moisture. The green weights do not show this great difference.

The gasoline treatment, on the other hand, did not show up the differences as early as the alcohol treatment did. After 2 months' growth, the plants on the treated soils still continued poorer than those on the untreated soil. About 10 weeks after seeding, the plants began to increase in growth on the treated soil.

It may be noted that on the Dunkirk clay loam, although all the 15 plants remained alive, only 9 plants grew to full height. It seems that for some reason the gasoline treatment had a retarding effect.

Conclusions Regarding Experiment III

A very marked increase in plant growth in favor of the alcohol treatment was noted in this test. The Volusia silt loam saturated yielded

better than the extracted treatment. With the Dunkirk clay loam the extracted treatment gave the highest yield. The gasoline treatment, as in Experiment I, shows no beneficial effect. In most cases it was detrimental.

Summary of Experiments I, II and III

1. The antiseptic treatment of soil in pots has a distinctly beneficial effect on the vegetative growth of succeeding oat and wheat crops. There is a slight advantage in favor of the saturated treatments.

2. Alcohol gave better results on plant growth than ether, benzene, toluene and gasoline, respectively. Gasoline is often harmful in its effects, both on plant growth and on germination. Ether seems to have little or no effect in either direction.

3. A stimulating influence of volatile antiseptics on plant growth occurs for both Dunkirk clay loam and Volusia silt loam, but in different degrees. Volusia silt loam, in general, responds the better.

TABLE IV

RESULTS OBTAINED WITH BUCKWHEAT GROWN ON SOILS PREVIOUSLY TREATED WITH VOLATILE ANTISEPTICS AND CROPPED TO OATS, EXPRESSED AS AVERAGE GREEN AND DRY WEIGHTS

(Planted August 5, 1915; harvested October 2, 1915)

Soil Type	Volatile Antiseptic	Untreated Soil			Saturated Soil			Extracted Soil		
			Weight of Dry Matter			Weight of Dry Matter			Weight of Dry Matter	
		Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.
DCL	Alcohol	34.25	7.90	100	35.75	8.10	102	32.60	7.9	100
[1]VSL	Alcohol	24.50	6.30	100	35.00	7.40	117	33.50	6.5	103
DCL	Benzene	34.00	8.20	100	40.50	9.30	101	40.00	9.3	113
DCL	Toluene	34.50	8.40	100	44.00	10.40	123	45.50	10.0	119
DCL	Gasoline	26.00	6.45	100	24.30	6.25	97	23.25	5.3	82
Grand Average		30.65	7.45	100	35.91	8.29	108	34.97	7.8	104

[1] In this experiment on the Volusia silt loam with the alcohol treatment, there was a set of soil fresh from the field. The following results were obtained: green weight of crop 15.5 gm. and dry weight 3.7 gm.

Experiment IV

The Effect of Volatile Antiseptics upon the Second Crop (Buckwheat) Grown after the Antiseptic Treatment of the Soil— Antiseptic: Alcohol, Benzene, Gasoline and Toluene—Crops: Oats and Buckwheat

The soil from Experiment I was taken from the pots, reworked, and the oat roots, as far as possible, removed. The soil was then replaced in each corresponding pot. On August 5, 1915, all the pots, except ether treatments, which were discarded, were seeded to buckwheat, 15 seeds to

a pot. The young plants were thinned to 10, when they were 10 inches high.

Observations were made of the growth of these plants. The seeds all germinated at the same time and the growth continued uniformly until about 5 weeks after seeding, when the plants on some of the treated soils showed a slightly better growth, as can be observed from the dry weights of Table IV. The buckwheat was harvested on October 2, 1915. Most of the seeds were ripe and some even ready to fall.

Conclusions Regarding Experiment IV

A residual effect of the antiseptic treatment of soil on plant growth is brought out distinctly in this experiment. It is not so marked, however, as was the direct influence shown in Experiments I, II and III. The saturated treatment again averages better than the extracted. In every case, except the benzene treatment, the saturated soil gave the higher yields. The gasoline treatment, although better on the saturated soil than on the extracted, gave in both cases lower yields than the untreated. If preference is to be given for the effectiveness in increasing crop yields, toluene seems to be slightly more effective than the other antiseptics. Gasoline is distinctly the least efficient. The Volusia silt loam soil responded to the treatment better than Dunkirk clay loam, as expressed in crop growth.

Experiment V

The Effect of Volatile Antiseptics upon the Second Crop (Oats) Grown After the Antiseptic Treatment of the Soil — Antiseptics: Alcohol, Ether and Toluene—Crops: Wheat and Oats

The soil after the harvesting of the wheat crop of Experiment II was taken out of the flower pots, reworked and transferred to ½-gallon glazed earthenware jars. Three hundred gm. of clean quartz sand were added to serve as a mulch. The moisture content was then kept constant until December 15, 1915, when the pots were seeded with oats. The oats were sterilized (52) with calcium hypochlorite powder, 10 gm. in 140 c.c. of water. Out of 20 seeds planted only 12 plants were left growing.

A notable effect here was that all of the plants on treated and untreated soils made the same progress in growth. This continued to be the case for the first two months of the experiment. After this period, however, the plants in the treated soil showed a distinct improvement over the plants in the untreated soil. Hardly any difference in growth was noticed between the saturated and the extracted soils. By the middle of March, 3 months after seeding, all the plants in the treated soil began to show a very healthy dark blue color, in contrast with those on the untreated pots, which did not have this vigorous appearance.

By the beginning of April there was not only a distinct difference in growth between the different treatments, but a difference was also ob-

served between the different types of soil. The Volusia silt loam invariably showed a better growth than the Dunkirk clay loam. This fact becomes easily apparent by a study of the results in dry weights given in Table V. In order that a fair comparison should be obtained between the different treatments, it was thought best to harvest the oats at this stage, although they had just begun to head.

TABLE V

RESULTS OBTAINED WITH OATS GROWN ON SOILS PREVIOUSLY TREATED WITH VOLATILE ANTISEPTICS AND CROPPED TO WHEAT, EXPRESSED IN AVERAGE GREEN AND DRY WEIGHTS

(Planted December 15, 1915; harvested April 17, 1916)

Soil Type	Volatile Antiseptic	Untreated Soil			Saturated Soil			Extracted Soil		
			Weight of Dry Matter			Weight of Dry Matter			Weight of Dry Matter	
		Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.	Wt. of Green Crop	gm.	Relative Wts.
DCL	Alcohol	8.90	2.35	100	29.05	6.45	274	30.65	6.15	261
VSL	Alcohol	35.60	6.80	100	44.90	9.15	135	40.95	8.60	126
DCL	Ether	8.85	2.05	100	23.20	4.85	236	23.65	4.45	217
VSL	Ether	26.50	5.40	100	30.10	5.70	105	27.95	5.60	104
DCL	Toluene	8.95	2.15	100	20.50	4.40	204	24.45	4.90	227
VSL	Toluene	23.70	5.00	100	48.65	9.50	190	39.35	7.90	158
Grand Average		18.75	3.79	100	32.73	6.67	190.6	31.16	6.26	182

Conclusions Regarding Experiment V

The residual effects from the antiseptic treatments on crop growth are brought out more markedly in this experiment than in the previous one. The saturated treatment again gave higher results than the extracted, except on the Dunkirk clay loam treated with toluene. The alcohol shows the highest benefit as measured in crop growth, with toluene next and ether last. There is only one exception to this order. The relative crop weights indicate a better response for the Dunkirk clay loam soil.

Summary of Experiments IV and V

1. A residual effect of the antiseptic treatment of soil upon the second crop is distinctly brought out in these experiments. The advantage is generally in favor of the saturated treatment.

2. The relative influence of the different antiseptics as measured by yields indicates that alcohol is most effective, and gasoline, the least.

3. The Volusia silt loam in general excels Dunkirk clay loam in its response to volatile antiseptics, as measured by the yield of the second crop after the antiseptic treatment.

Experiment VI

The Effect of the Alcoholic Extract of Soils upon Oats Grown in Water Cultures

The object in this experiment was to study the effect on plant growth of the residue of the alcoholic extract obtained in Experiment III. For this purpose the alcoholic residue obtained from recovering the alcohol extract of both Dunkirk clay loam and Volusia silt loam was evaporated to dryness on water bath. It was then taken up three consecutive times with 70 per cent alcohol, digested and filtered each time. Finally the residue was taken up with distilled water previously treated with carbon black.

The water-soluble portion of this alcoholic soluble material was then determined. From the Dunkirk clay loam soil 0.5 gm. was derived, and from the Volusia silt loam soil 1.1 gm., the alcoholic-soluble residue being, respectively 2 and 3.1 gm. This water-soluble residue was used in growing oats in the following way. Half of this water filtrate of both types of soil was added in the proportions of 5, 50 and 100 parts per million, respectively, to a full nutrient solution of the following composition:

COMPOSITION OF NUTRIENTS

$Ca(NO_3)_2$	2.70 gm.
$MgSO_4$	0.60 gm.
KCl	0.75 gm.
KH_2PO_4	1.50 gm.
$FeSO_4$	0.05 gm.
Distilled H_2O	10.00 liters

Full nutrient solutions served as checks to the above. The other half of the extract was added in the same proportions as above to carbon-black-treated distilled water. Distilled water solutions served as checks in this case. Oat seedlings were grown in these solutions for one month, all of the solutions being replaced once during this period. Four plants as uniform as it was possible to obtain were grown in each 8-ounce bottle.

Conclusions Regarding Experiment VI

From the data it seems that a soluble substance has been removed from the soil, which in water culture, is detrimental to plant growth. Its injurious effect in all cases with one exception was brought out more in the presence of nutrients than with distilled water alone. The toxic effect was not apparent in the soil itself as has already been shown in previous experiments; if it were, its influence would have been noticed in the comparison of the saturated and extracted treatments of Experiments I, II and III. As this was not the case, the results seem to substantiate the work of other investigators in their conclusion that a substance, toxic when in water culture, may not be toxic in the soil itself.

TABLE VI

RESULTS OBTAINED WITH OATS GROWN IN WATER CULTURE AND TREATED WITH WATER-SOLUBLE ALCOHOLIC EXTRACT FROM SOIL, EXPRESSED AS GREEN AND DRY WEIGHTS

(Plants grown for one month

Soil Type	Treatment	Check			5 p. p. m. of Extract			50 p. p. m. of Extract			100 p. p. m. of Extract		
		Weight Green Crop	Weight of Dry Matter		Weight Green Crop	Weight of Dry Matter		Weight Green Crop	Weight of Dry Matter		Weight Green Crop	Weight of Dry Matter	
			gm.	Rel. Wts.		gm.	Rel. Wts.		gm.	Rel. Wts.		gm.	Rel. Wts.
VSL	Distilled H_2O	1.3925	0.1837	100	1.3923	0.1750	95	1.3949	0.1656	90	1.2689	0.1648	88
VSL	Full Nutrient	5.7888	0.7005	100	3.3616	0.4154	59	3.4100	0.4499	64	3.4962	0.4840	69
DCL	Distilled H_2O	1.4340	0.1917	100	.....	.....	..	0.9601	0.1107	57	1.3250	0.1680	87
DCL	Full Nutrient	4.8863	0.7788	100	3.3167	0.4773	61	2.7311	0.4705	60	1.7294	0.3813	48
Grand Aver.		3.3754	0.4637	100	2.3569	0.3559	71.6	2.1240	0.2992	67.7	1.9549	0.2995	73

IV. Laboratory Investigations

Experiment VII

The Effect of Alcohol and Toluene Treatments on Ammonification and Nitrification in the Soil and upon the Total Soluble Salts after Incubation for Periods of 3, 6 and 12 Weeks

The effect of the alcohol and toluene treatments on the ammonifying and nitrifying power of the soil and upon the total water-soluble salts after periods of 3, 6 and 12 weeks, was first taken up. For this a portion of the alcohol and toluene-treated soils described under Experiment II was utilized directly after the treatment with the volatile antiseptics. Nitrates and total soluble salts were determined from duplicate samples of each treatment. Ammonia was determined from other duplicate samples.

TABLE VII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH ALCOHOL, EXPRESSED AS PARTS PER MILLION

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
NITRIC NITROGEN						
After 3 weeks	8.25	100	Nil	Nil	Nil	Nil
After 6 weeks	25.60	310	Nil	Nil	Nil	Nil
After 12 weeks	61.00	739	2.3	28	1.65	20
AMMONIACAL NITROGEN[1]						
After 3 weeks	11.05	100	24.1	218	22.90	207
After 6 weeks	70.00	633	109.3	965	193.10	1748
After 12 weeks	16.80	152	65.4	588	94.50	855
TOTAL WATER-SOLUBLE SALTS						
After 3 weeks	64	100	75	117	73	114
After 6 weeks	62	97	72	113	70	109
After 12 weeks	78	122	83	130	77	120

[1] By ammoniacal nitrogen is meant ammonia expressed as nitrogen.

Conclusions Regarding Experiment VII

The antiseptic treatment exerts a definite effect on the nitrifying and ammonifying processes of the soil. The nitrifying power was practically inhibited by the alcohol and toluene treatments during the first 6 weeks, but at the end of 12 weeks small amounts were found. During the same period, there was a gradual increase of ammonia reaching its maximum at the end of 6 weeks followed by some depression.

The difference between the saturated and extracted treatments varied, but not to a marked extent. The Dunkirk clay loam soil responded more

TABLE VIII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM, WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH ALCOHOL, EXPRESSED IN PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
After 3 weeks	4.0	100	Nil	Nil	Nil	Nil
After 6 weeks	9.6	240	Nil	Nil	Nil	Nil
After 12 weeks	44.5	1112	1.85	46	2.2	55

AMMONIACAL NITROGEN

After 3 weeks	22.4	100	27.3	122	21.5	96
After 6 weeks	96.2	429	109.3	487	172.5	769
After 12 weeks	24.7	110	39.6	177	44.9	200

TOTAL WATER-SOLUBLE SALTS

After 3 weeks	121	100	128	106	99	82
After 6 weeks	125	103	130	107	116	96
After 12 weeks	130	107	85	70	76	63

TABLE IX

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH TOLUENE, EXPRESSED AS PARTS PER MILLION OF DRY SOIL

NITRIC NITROGEN

Time	Untreated		Toluene Saturated		Toluene Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
After 3 weeks	8.30	100	Nil	Nil	Nil	Nil
After 6 weeks	38.75	467	Trace	Trace	Nil	Nil
After 12 weeks	59.05	711	70.25	845	3.20	39

AMMONIACAL NITROGEN

After 3 weeks	18.90	100	25.30	134	34.90	185
After 6 weeks	68.60	363	132.05	698	132.10	698
After 12 weeks	5.50	28	12.35	64	33.15	175

TOTAL WATER-SOLUBLE SALTS

After 3 weeks	53	100	52	98	51	96
After 6 weeks	62	117	65	123	57	107
After 12 weeks	80	151	83	156	57	107

to the treatment than Volusia silt loam. The toluene antiseptic seems not quite as effective as the alcohol. The general results compare closely.

That these data corroborate the results of Russell and Hutchinson (43) and most other investigators can be seen from the literature, viz: that nitrification is inhibited, and ammonification gradually increased for a certain time. The latter then gradually decreases or remains constant, while the former is later stimulated.

TABLE X

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH TOLUENE, EXPRESSED IN PARTS PER MILLION

Time	Untreated		Toluene Saturated		Toluene Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
NITRIC NITROGEN						
After 3 weeks	4.1	100	Nil	Nil	Nil	Nil
After 6 weeks	17.2	420	Trace	Trace	Trace	Trace
After 12 weeks	48.6	1180	2.6	63	1.9	46
AMMONIACAL NITROGEN						
After 3 weeks	20.6	100	25.3	124	26.2	127
After 6 weeks	101.1	49	122.6	610	130.6	660
After 12 weeks	19.4	94	39.7	193	33.7	164
TOTAL WATER-SOLUBLE SALTS						
After 3 weeks	121	100	145	120	133	110
After 6 weeks	124	103	137	114	127	105
After 12 weeks	129	107	135	111	121	100

There is a general tendency for the total water-soluble salts to increase correspondingly to the duration of incubation. The general influence of the treatments with volatile antiseptics seems to be slightly to increase water-soluble salts. No definite difference can be noted between different soils or between the two methods of applying the antiseptic. The data regarding water-soluble salts are variable.

Experiment VIII

The Direct Effect of Alcoholic and Gasoline Treatments on Ammonification and Nitrification in the Soil and upon Total Soluble Salts Incubation for Periods of 2, 4 and 6 Months

For this investigation, definite quantities were taken of the same soil as that used in Experiment III. The incubation periods were 2, 4 and 6 months, respectively.

The incubation temperature during this time varied from 18° to 26° C. The experiments with the alcohol treatment was begun on January 26, 1916, whereas with the gasoline-treated soils incubation was started on February 22.

TABLE XI

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH ALCOHOL, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
After 2 months	6.70	100	Nil	Nil	Nil	Nil
After 4 months	50.50	753	Nil	Nil	Nil	Nil
After 6 months	59.40	886	29.20	435	46.3	691

AMMONIACAL NITROGEN

After 2 months	5.75	100	23.00	400	19.3	335
After 4 months	6.45	112	13.40	233	8.0	139
After 6 months	Trace	Trace	1.25	21.7	Trace	Trace

TOTAL WATER-SOLUBLE SALTS

After 2 months	104	100	106	102	122	117
After 4 months	132	127	112	108	113	108
After 6 months	146	140	157	151	115	110

TABLE XII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH ALCOHOL, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
After 2 months	19.2	100	Nil	Nil	Nil	Nil
After 4 months	24.8	129	Nil	Nil	Nil	Nil
After 6 months	60.6	314	4.8	25	Trace	Trace

AMMONIACAL NITROGEN

After 2 months	5.9	100	23.8	403	23.8	403
After 4 months	Trace	Trace	11.4	193	12.3	209
After 6 months	Trace	Trace	7.6	129	11.0	186

TOTAL WATER-SOLUBLE SALTS

After 2 months	170	100	78	46	72	43
After 4 months	168	99	72	43	79	46
After 6 months	194	114	88	52	79	46

TABLE XIII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH ALCOHOL, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Gasoline Saturated		Gasoline Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
After 2 months	7.50	100	Trace	Trace	Trace	Trace
After 4 months	21.40	285	8.9	119	7.7	103
After 6 months	24.80	331	20.5	273	19.2	256

AMMONIACAL NITROGEN

After 2 months	7.47	100	15.2	203	15.2	203
After 4 months	Trace	Trace	Trace	Trace	Trace	Trace
After 6 months	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

After 2 months	167	100	121	72	123	74
After 4 months	136	81	137	82	123	74
After 6 months	120	72	116	69	103	62

TABLE XIV

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM WITHOUT TREATMENT AND SUBSEQUENT TO TREATMENT WITH GASOLINE, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Gasoline Saturated		Gasoline Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
After 2 months	16.8	100	Trace	Trace	Trace	Trace
After 4 months	51.1	304	11.3	67	18.2	108
After 6 months	15.5	92	198.0	118	196.0	118

AMMONIACAL NITROGEN

After 2 months	9.2	100	14.3	155	12.8	139
After 4 months	Trace	Trace	11.4	124	Trace	Trace
After 6 months	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

After 2 months	180	100	170	94	166	92
After 4 months	168	93	161	89	177	98
After 6 months	176	97	166	92	170	94

Conclusions Regarding Experiment VIII

The general results shown by this test verify those of Experiment VII. It should be remembered that this experiment ran for a period twice as long as the previous one and that certain differences may be attributed to that fact.

Nitrification was inhibited for a certain time for the alcohol and gasoline treatments. This influence endured longer for the former than for the latter. The ammonifying process must have previously reached its maximum point according to the findings of Experiment VII, and was gradually decreasing.

As in Experiment VII, there is a general tendency for the soluble matter to increase with duration of incubation. No very definite conclusions can be drawn as to the effects from the different antiseptics or to differences between the two soils.

Experiment IX

The Effect of Volatile Antiseptics upon Nitrification and Ammonification in the Soil and upon the Total Water-Soluble Salts, the Soil Having been Cropped to Oats (Experiment I) and to Buckwheat (Experiment IV) Subsequent to the Antiseptic Treatment

After harvesting the crop of Experiment IV the soil was taken from the pots, reworked and maintained in a loose structural condition during incubation. Tests were made at the beginning and after 14 days for nitrates, ammonia and total soluble salts with the results given in Tables XV to XIX.

TABLE XV

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATE AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH ALCOHOL AND CROPPED TO OATS, AND THEN TO BUCKWHEAT, JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
NITRIC NITROGEN						
At beginning	4.50	100	14.2	316	3.9	87
After 14 days	17.30	384	17.1	380	20.9	464
AMMONIACAL NITROGEN						
At beginning	17.95	100	4.8	27	4.2	23
After 14 days	9.80	55	4.1	23	4.3	24
TOTAL WATER-SOLUBLE SALTS						
At beginning	132	100	133	101	119	90
After 14 days	112	85	100	76	134	102

TABLE XVI

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM PREVIOUSLY TREATED WITH ALCOHOL AND CROPPED TO OATS AND THEN TO BUCKWHEAT JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	3.9	100	7.2	185	9.4	241
After 14 days	7.5	192	18.7	480	22.2	570
AMMONIACAL NITROGEN						
At beginning	4.3	100	5.7	132	12.9	300
After 14 days	10.3	240	8.7	202	7.3	170
TOTAL WATER-SOLUBLE SALTS						
At beginning	52	100	68	131	70	135
After 14 days	64	123	102	196	94	181

TABLE XVII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH TOLUENE AND CROPPED TO OATS AND THEN TO BUCKWHEAT JUST PRIOR TO TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Toluene Saturated		Toluene Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	7.0	100	15.7	224	16.80	240
After 14 days	10.8	154	14.9	213	16.20	231
AMMONIACAL NITROGEN						
At beginning	5.3	100	5.5	104	4.15	77
After 14 days	3.7	70	4.0	75	2.80	53
TOTAL WATER-SOLUBLE SALTS						
At beginning	141	100	227	161	193	137
After 14 days	137	97	231	164	210	149

TABLE XVIII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH BENZENE AND CROPPED TO OATS AND THEN TO BUCKWHEAT JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Benzene Saturated		Benzene Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	5.9	100	6.0	102	6.20	105
After 14 days	8.6	146	14.0	237	10.05	169
AMMONIACAL NITROGEN						
At beginning	6.5	100	5.1	78	3.10	48
After 14 days	Trace	Trace	8.4	129	11.70	180
TOTAL WATER-SOLUBLE SALTS						
At beginning	130	100	144	122	136	115
After 14 days	134	113	129	109	151	128

TABLE XIX

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH GASOLINE AND CROPPED TO OATS AND THEN TO BUCKWHEAT JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Gasoline Saturated		Gasoline Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	17.80	100	12.80	72	12.80	72
After 14 days	17.80	100	23.50	132	24.80	139
AMMONIACAL NITROGEN						
At beginning	3.95	100	4.20	106	4.15	105
After 14 days	9.20	233	3.15	80	4.10	104
TOTAL WATER-SOLUBLE SALTS						
At beginning	148	100	317	214	301	204
After 14 days	177	119	242	163	224	151

Conclusions Regarding Experiment IX

The data of this experiment indicate that the residual effect of the antiseptics is very low, a condition which should be expected after so long a period. Between the saturated and extracted treatments no marked difference is found, either as to nitrification or ammonification. There was a general tendency for a decrease in ammonification the longer the incubation was carried on.

The marked difference previously observed between the two types of soil from the direct treatment already discussed were lacking. The total soluble salts either remained constant or showed a tendency to increase with incubation.

Experiment X

The Effect of Volatile Antiseptics upon Nitrification and Ammonification in the Soil and upon the Total Water-Soluble Salts, the Soil Having been Cropped to Wheat (Experiment II) and to Oats (Experiment V) Subsequent to the Antiseptic Treatment

In Experiment V it has been stated that one pot of each soil was kept for chemical study after harvesting the oats which followed the wheat of Experiment II. This study was carried out by keeping these pots in the laboratory and making determinations for nitrates, ammonia and total soluble salts at the beginning, after 20 days and finally, at the end of 60 days. Samples were taken from these pots with a cork borer of 2 cm. diameter. The holes thus made were filled up with clean sand and the moisture content kept constantly at 25 per cent.

TABLE XX

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH ALCOHOL AND CROPPED TO WHEAT AND THEN TO OATS JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
NITRIC NITROGEN						
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 20 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil
AMMONIACAL NITROGEN						
At beginning	3.5	100	5.3	151	5.2	149
After 20 days	3.8	108	5.3	151	5.3	151
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil
TOTAL WATER-SOLUBLE SALTS						
At beginning	42	100	45	107	51	122
After 20 days	38	91	39	93	46	109
After 60 days	47	112	45	107	46	109

TABLE XXI

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM PREVIOUSLY TREATED WITH ALCOHOL AND CROPPED TO WHEAT AND THEN TO OATS JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 20 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

AMMONIACAL NITROGEN

At beginning	5.2	100	5.4	104	5.5	106
After 20 days	5.3	102	5.2	100	5.3	102
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

At beginning	41	100	42	102	42	102
After 20 days	44	107	44	107	37	90
After 60 days	39	95	56	137	60	146

TABLE XXII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH ETHER AND CROPPED TO WHEAT AND THEN TO OATS JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Ether Saturated		Ether Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 20 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

AMMONIACAL NITROGEN

At beginning	3.8	100	2.5	66	2.6	68
After 20 days	5.2	137	5.3	139	5.4	142
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

At beginning	33	100	37	112	39	118
After 20 days	37	112	46	139	47	142
After 60 days	51	154	65	197	45	136

TABLE XXIII

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM PREVIOUSLY TREATED WITH ETHER AND CROPPED TO WHEAT AND THEN TO OATS JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Ether Saturated		Ether Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 20 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

AMMONIACAL NITROGEN

At beginning	5.42	100	2.63	48	2.57	47
After 20 days	5.56	103	5.46	101	2.93	54
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

At beginning	47	100	45	96	46	98
After 20 days	47	100	41	87	43	91
After 60 days	39	83	72	153	60	128

TABLE XXIV

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM PREVIOUSLY TREATED WITH TOLUENE AND CROPPED TO WHEAT AND THEN TO OATS JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Toluene Saturated		Toluene Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 20 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

AMMONIACAL NITROGEN

At beginning	3.54	100	5.1	144	5.1	144
After 20 days	2.41	68	5.2	147	5.2	147
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

At beginning	45	100	51	113	42	93
After 20 days	47	104	47	104	41	91
After 60 days	47	104	51	113	54	120

Following the determinations tabulated above, the soils were taken from the pots, reworked and maintained in loose structural condition during the incubation tests. After 20 days determinations were made for nitrates, ammonia and total water-soluble salts. The results therefrom are not reported in tabular form. No nitrates were found in any of the different treatments, nor was any ammonia found with toluene treatment in either type of soil. In case of the alcohol and ether treatments traces of ammonia were found. The total soluble salts hardly varied from the determinations reported at the end of 60 days on the soil as it stood in the pots.

TABLE XXV

TOTAL WATER-SOLUBLE SALTS AND WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM PREVIOUSLY TREATED WITH TOLUENE AND CROPPED TO WHEAT AND THEN TO OATS JUST PRIOR TO THE TESTS, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Toluene Saturated		Toluene Extracted	
	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts	Actual Amounts	Relative Amounts
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 20 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

AMMONIACAL NITROGEN

At beginning	2.5	100	3.2	128	3.5	140
After 20 days	1.9	76	2.5	100	2.5	100
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil

TOTAL WATER-SOLUBLE SALTS

At beginning	48	100	50	104	41	85
After 20 days	48	100	51	106	41	85
After 60 days	50	104	53	110	46	96

Conclusions Regarding Experiment X

As in Experiment IX, the chemical results indicate little difference between the residual saturated or extracted treatments with volatile antiseptics. The results from the treatments by the two methods are very similar to those obtained with the untreated soil. This holds true for both nitrification and ammonification. The period of incubation did not alter either the nitrifying or the ammonifying results. In Tables XXII and XXIII there is a slight tendency during the first two weeks towards an increase in ammonification with ether as the antiseptic. This was inhibited, however, at the end of 60 days. The total water-soluble salts did not materially change during the process of incubation. No residual effect, of the volatile antiseptics, developed in either soil type.

Experiment XI

Effect of the Physical Condition of Soil Cropped to Oats (in Experiment III), Subsequent to a Treatment with Volatile Antiseptics, upon Total Soluble Salts and upon Water-Soluble Nitrogen Occurring as Nitrates and Ammonia

In order to determine what effect the physical condition of the soil would have upon the nitrifying and ammonifying power of the soil, the following test was carried out. Of the duplicate treatments of each type of soil after harvesting the crop of Experiment III, one pot was maintained undisturbed and determinations made at the beginning, after 21 days and after 35 days, for gasoline-treated soils, and after 21 and 60 days for alcohol-treated soils. The duplicate pot of soil in each case was reworked and maintained in good tilth for the same period of time as above, and is designated in the tables as incubated soil.

TABLE XXVI

EFFECT OF PHYSICAL CONDITION OF SOIL ON TOTAL WATER-SOLUBLE SALTS AND UPON WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM CROPPED TO OATS (EXPERIMENT III) AND SUBSEQUENT TO A TREATMENT WITH ALCOHOL, EXPRESSED AS PARTS PER MILLION

NITRIC NITROGEN

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 21 days	0.99	2.4	Trace	0.54	Trace	0.64
After 60 days	14.20	36.7	2.1	4.60	3.7	5.40
AMMONIACAL NITROGEN						
At beginning	5.1	Nil	5.1	Nil	5.2	Nil
After 21 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil
TOTAL WATER-SOLUBLE SALTS						
At beginning	85	85	84	86	84	85
After 21 days	146	144	112	112	84	84
After 60 days	120	123	56	55	48	46

Conclusions Regarding Experiment XI

These data indicate that the effect of the antiseptics has been inhibitive. At the beginning of incubation, regardless of differences of soil or in treatment, there was no difference to be seen in the nitrifying or ammonifying processes of the soil. The nitrates and ammonia may have been entirely used up by the plants. Even between the two types of soil there was no marked difference to be noticed. The Volusia silt loam shows a higher yield of nitrates for gasoline treatment. The main point

TABLE XXVII

EFFECT OF PHYSICAL CONDITION OF SOIL ON TOTAL WATER-SOLUBLE SALTS AND UPON WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM CROPPED TO OATS (EXPERIMENT III) AND SUBSEQUENT TO A TREATMENT WITH ALCOHOL, EXPRESSED AS PARTS PER MILLION

Time	Untreated		Alcohol Saturated		Alcohol Extracted	
	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated
NITRIC NITROGEN						
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 21 days	6.6	7.5	Trace	1.1	Trace	1.6
After 60 days	13.3	13.2	1.5	3.0	2.8	3.7
AMMONIACAL NITROGEN						
At beginning	5.1	5.1	5.1	5.1	5.0	5.0
After 21 days	Nil	Nil	Nil	Nil	Nil	Nil
After 60 days	Nil	Nil	Nil	Nil	Nil	Nil
TOTAL WATER-SOLUBLE SALTS						
At beginning	44	45	62	68	46	48
After 21 days	85	85	80	84	86	85
After 60 days	52	54	57	59	50	52

TABLE XXVIII

EFFECT OF PHYSICAL CONDITION OF SOIL ON TOTAL WATER-SOLUBLE SALTS AND UPON WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN DUNKIRK CLAY LOAM CROPPED TO OATS (EXPERIMENT III) AND SUBSEQUENT TO A TREATMENT WITH GASOLINE, EXPRESSED AS PARTS PER MILLION

Time	Untreated		Gasoline Saturated		Gasoline Extracted	
	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated
NITRIC NITROGEN						
At beginning	Trace	Trace	Nil	Nil	Nil	Nil
After 21 days	Trace	3.5	Trace	3.5	Trace	3.6
After 35 days	2.4	8.0	2.6	7.6	3.0	8.5
AMMONIACAL NITROGEN						
At beginning	Nil	Nil	Nil	Nil	Nil	Nil
After 21 days	Nil	Nil	Nil	Nil	Nil	Nil
After 35 days	Nil	Nil	Nil	Nil	Nil	Nil
TOTAL WATER-SOLUBLE SALTS						
At beginning	82	82	88	88	92	94
After 21 days	80	84	74	77	73	82
After 35 days	82	88	76	102	74	99

to be brought out in this experiment is that the physical condition of the soil can be largely eliminated as a factor influencing the nitrifying and ammonifying processes of the soil for the conditions under which these experiments were carried out. The Volusia silt loam treated with gasoline shows greater increase in the nitrifying power of the soil than the corresponding alcohol series.

TABLE XXIX

EFFECT OF PHYSICAL CONDITION OF SOIL ON TOTAL WATER-SOLUBLE SALTS AND UPON WATER-SOLUBLE NITROGEN OCCURRING AS NITRATES AND AS AMMONIA IN VOLUSIA SILT LOAM CROPPED TO OATS (EXPERIMENT III) AND SUBSEQUENT TO A TREATMENT WITH GASOLINE, EXPRESSED AS PARTS PER MILLION

Time	Untreated		Gasoline Saturated		Gasoline Extracted	
	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated	Actual Amounts in Pots	Actual Amounts in Soil Incubated
NITRIC NITROGEN						
At beginning	2.4	2.4	2.8	2.8	Nil	Nil
After 21 days	3.7	19.0	11.3	18.5	12.0	19.3
After 35 days	7.9	22.7	25.3	36.2	30.0	39.5
AMMONIACAL NITROGEN						
At beginning	6.3	6.2	8.2	8.2	6.5	6.6
After 21 days	Nil	Nil	Nil	Nil	Nil	Nil
After 35 days	Nil	Nil	Nil	Nil	Nil	Nil
TOTAL WATER-SOLUBLE SALTS						
At beginning	35	35	50	50	40	39
After 21 days	50	52	52	80	72	100
After 35 days	65	85	89	102	55	106

Experiment XII

Development of Acid in Soil Due to the Action of Alcohol

The idea was suggested that volatile antiseptics applied to the soil might be oxidized with the formation of an acid. If an acid were formed, it might not only have a depressing effect on the soil organisms but might liberate certain mineral elements in the soil. The effects of volatile antiseptics upon crop growth and bacterial action might thus at least partially be accounted for. In order to test this out, the following experiment was performed.

About 250 gm. of both Dunkirk clay loam and Volusia silt loam were used, separate samples being placed in 2-inch glass cylinders. The various samples were then saturated over night with distilled water, potassium nitrate and 70 per cent alcohol, respectively. The next morning, more water was added to the soils saturated with distilled water and potassium nitrate until about 150 c.c. of solution had percolated through. The

alcohol-saturated soil was treated similarly but with the concentration of alcohol previously used. After the initial percolations were obtained, the soils were spread out for 3 days and allowed to dry. They were then percolated again as already described. This second percolate obtained from the soils is designated in the table of data as "after aeration."

Fifty-c.c. quantities of the various percolates were diluted to 200 c.c. with CO_2-free water and titrated against N/10 NaOH. The results reported in Table XXX are the average of duplicate determinations.

TABLE XXX

ACTUAL AND RELATIVE AMOUNTS OF ACID DEVELOPED IN SOIL DUE TO TREATMENT OF ALCOHOL

Type Soil	Percolate	Water		Potassium Nitrate		Alcohol	
		Actual Amts. c.c.	Rel. Amts.	Actual Amts. c.c.	Rel. Amts.	Actual Amts. c.c.	Rel. Amts.
DCL	Initial percolation	0.15	100	0.65	430	0.60	400
DCL	After aeration	0.20	100	0.70	350	0.70	350
VSL	Initial percolation	0.20	100	5.50	2750	0.70	350
VSL	After aeration	0.25	100	0.65	260	0.75	300

Conclusions Regarding Experiment XII

In the case of Dunkirk clay loam, a soil neutral or very slightly acid, the relative amounts of acid as developed by KNO_3 and alcohol in the first percolation are about the same. With the Volusia silt loam, however, a soil intensely acid, the KNO_3 in the case of the initial percolate generates an acidity several times that of distilled water. The alcohol, on the other hand, exhibits about the same results as when percolated through the Dunkirk clay loam. It seems evident that the acidity developed by the alcohol is about at its maximum in both cases.

The second percolate of the soils shows an acidity for the alcohol in both cases lower than in the initial trial. If the antiseptic forms an acid to any degree, the drying action should be expected to augment the acid condition.

From the fact, therefore, (a) that the alcohol reacted about the same for both soils, (b) that the second percolate showed an actual lowering acidity in spite of the aeration of the soil, and (c) that the aeration of the alcohol-treated soils was so low as probably to be within experimental error, it seems impossible to conceive that the development of acids by the action of the antiseptic could be an important factor in influencing plant and bacterial action, especially in the magnitude already described in the preceding experiments.

V. Summary

1. The application of volatile antiseptics to the soils used in this investigation gave beneficial results on the crops subsequently grown thereon.

2. A beneficial, residual effect is observed for the second crop after the application of the volatile antiseptics. This, however, was in all cases less marked than with the first cropping. Both types of soils responded to treatment, but somewhat differently.

3. The volatile antiseptics experimented with had a definite effect upon the ammonification and nitrification of the soil, enhancing the former and inhibiting the latter. There is a tendency for the volatile antiseptics to increase the water-soluble salts of the soil.

4. The effect of the antiseptics upon the ammonifying and nitrifying processes of the soils after two crops were grown seems to disappear.

5. No marked differences were observed as to plant growth and biological activity between the saturation and extraction methods of applying the volatile antiseptics to the soil.

6. In these experiments the physical condition of the soil as indicated by its ammonification and nitrification does not seem to be the cause of the influences noted upon plant growth and bacterial action.

7. By the extraction of soil with alcohol, a substance was removed which was toxic in water cultures but not at all toxic when in the soil itself.

8. The development of acids in the soil as a result of some action or change of the alcohol was found to be too slight to account for the marked effects of volatile antiseptics upon plant growth and bacterial action.

Final Conclusions

The beneficial influences obtained by treating the soil with volatile antiseptics can not be ascribed to a change in physical condition, to a suppression of some toxic material, or to a development of acids from the action of the antiseptics. The method of applying the antiseptics seems to have no marked influence upon the results obtained.

The closely coordinated stimulation of plant and bacterial activity due to the treatment of the soil with volatile antiseptics points strongly towards a biological interpretation, with due regard for the chemical considerations, of the effects therefrom.

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PLATE I

The effect of alcohol and gasoline treatments of Dunkirk clay loam on the growth of oats at the blooming stage.
(For data see Table III.)

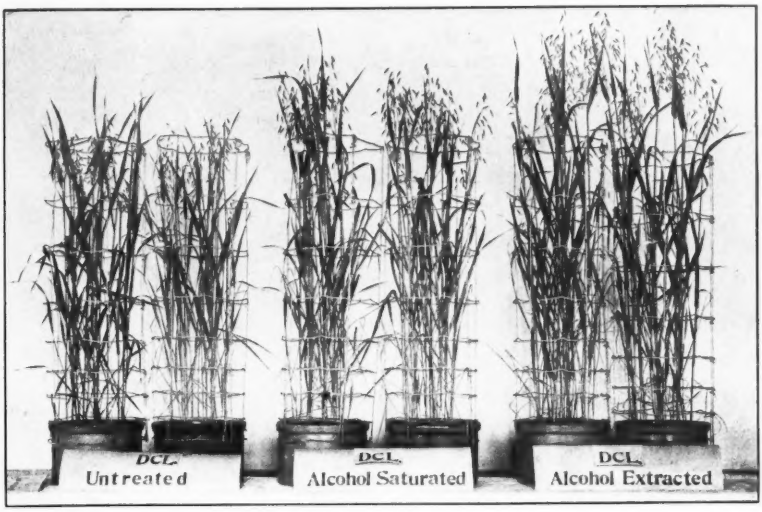
DCL
Untreated
DCL
Alcohol Saturated
DCL
Alcohol Extracted

Fig. 1

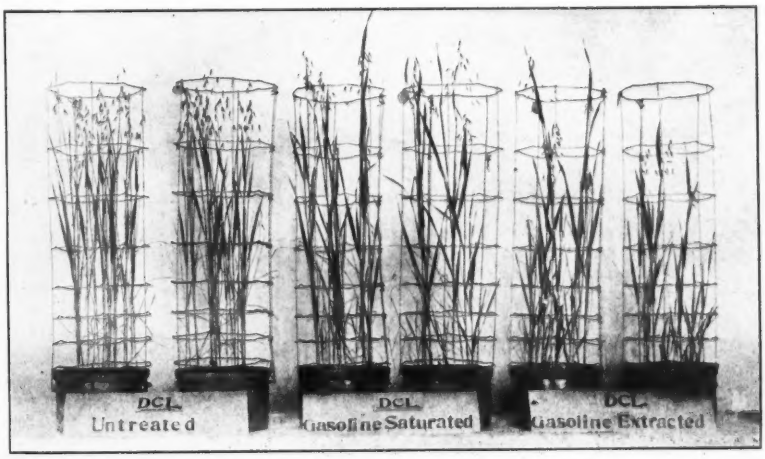
DCL
Untreated
DCL
Gasoline Saturated
DCL
Gasoline Extracted

Fig. 2

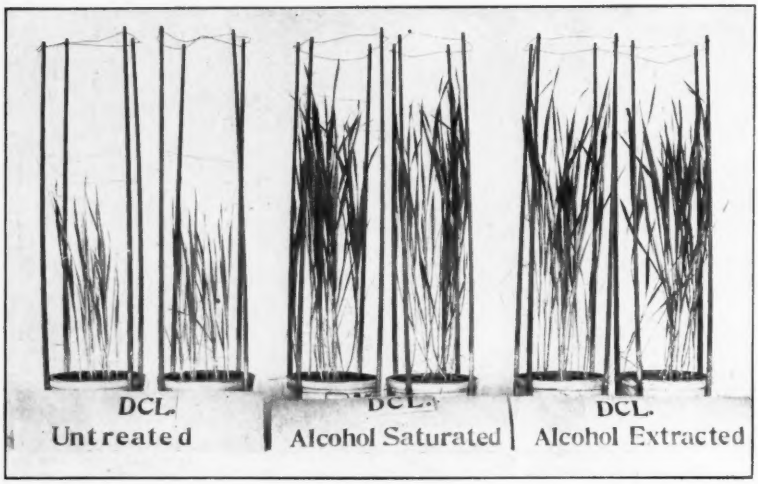
DCL.
Untreated
DCL.
Alcohol Saturated
DCL.
Alcohol Extracted

Fig. 1

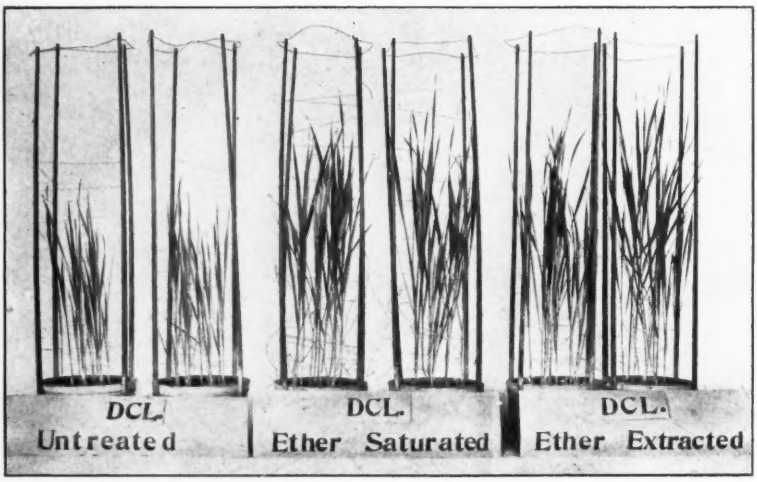
DCL.
Untreated
DCL.
Ether Saturated
DCL.
Ether Extracted

Fig. 2

PLATE II

The effect of alcohol and ether treatments of Dunkirk clay loam soil on the growth of oats just before heading.
(For data see Table V.)

THE INFLUENCE OF SOIL TEMPERATURE UPON SEEDLING CORN[1]

By

Byron D. Halsted, *Botanist, New Jersey Agricultural Experiment Station*, and Selman A. Waksman,[2] *Fellow in Physiology, University of California*

Introduction

Two fully comparable lots of corn were subjected to different temperatures in a greenhouse, namely, (1) the warmth of the bed in mid-summer, from July 30 to August 26, and (2) the comparatively cool condition of the same bed from October 29 to November 29 before the furnace fire was started.

The temperature of the soil was taken twice daily, 6 a. m. and 6 p. m.,[3] during the two series of tests, with the results in averages for each series as shown below.

	6 a. m.	6 p. m.	Daily Averages	Range
Summer Series	21.85°C.	29.52°C.	25.68°C.	17-34°C.
Autumn Series	10.35°C.	15.32°C.	12.83°C.	5.5-21°C.
Differences	11.50°C.	14.20°C.	12.85°C.	12.5-13°C.

It is seen that the thermometrical records for the summer series averaged nearly twice as far above the freezing point as those for the autumn tests.

It is, of course, true that the length of day was a varying environmental factor, but the daily loss of light for the autumn series was compensated for by the greater length of time allowed for this test.

The Subjects

The tests involved the factors of (1) texture, and (2) size of grain, and these were obtained by the selection of the crosses that carried upon the same ears both starchy and sugary grains, as follows:

1. Golden Nugget upon Adams Crosby F_2; a large starchy upon a medium-sized sugary kind.
2. Voorhees Red upon Eight-rowed Pop F_2; a medium sugary upon a large pop (starchy) kind.
3. Voorhees Red Stowell upon Golden Queen F_2; a large sugary upon a small pop (starchy) kind.

Only enough ears were taken, to yield the required number of sugary grains of each cross, thus limiting, as nearly as possible, an otherwise wide range of parentage.

[1] Received for publication December 7, 1916.

[2] This paper was prepared when the junior author was Research Assistant at the New Jersey Agricultural Experiment Station.

[3] These daily records yield averages that are somewhat below the absolute.

The shelled corn was next assorted into the: (1) starchy, and (2) sugary grains, and these again separated into the (3) larger and (4) smaller kernels, all defective kernels having been discarded.

When ready for planting, there were in duplicate the following sets of the grains, all units having 250 kernels: (1) starchy larger, (2) starchy smaller, (3) sugary larger, (4) sugary smaller, making a total of 3000 grains each for the summer and the autumn tests.

Planting, Care and Harvesting

The soil of the greenhouse bed was of good quality, and the grains were planted uniformly 2 inches deep, in drills, with 8 square inches of surface for each grain.

The beds were cared for alike, in the usual manner as to watering, ventilation, protection, etc.

Records of emergence of seedlings from the soil were taken twice a day, at 6 a. m. and 6 p. m., and the harvest consisted of the careful removal of the plants from the soil, the measuring of the mesotyl and the whole length of each plant not including the roots. The weights were taken of (1) the grains of each of the several groups, and (2) their seedlings.

From these records the viability of the seed and vigor and variability have been computed, as shown in the following series of tables.

TABLE I

WEIGHT OF SEED, AS RELATED TO TEXTURE AND SIZE OF GRAIN

	Averages gm.	Starchy gm.	Sugary gm.	Larger gm.	Smaller gm.
1. Gold Nugget—Crosby	.331	.380	.280	.375	.285
2. Voorhees Red—Eight-rowed Pop....	.211	.230	.190	.237	.185
3. Voorhees Stowell—Golden Queen....	.224	.245	.200	.247	.197
Averages	.256	.285	.223	.286	.222

In Table I it is shown that the Gold Nugget-Crosby was over 60 per cent larger than the other two kinds and here the difference between the starchy and sugary grains was the greatest, being nearly 25 per cent. The same wide difference between the large and small is seen here, while the two crosses with pop corn show far less range.

From the averages it is seen that accidentally the group of starchy grains weighs practically the same as that of the larger grains used in the test. Likewise, the sugary and smaller groups agree closely in weights. The two sets of the lighter weights differ from their heavier associates by nearly 22 per cent.

It is noted in Table II that the difference in viability is very great between the summer and autumn-grown crops (63.44 per cent) and in favor of the warm temperature.

It is seen, that there are decided differences in viability among the crosses, and that the rank is maintained throughout the table, excepting in the column for summer grown, where the second and third rank change places.

TABLE II

VIABILITY, AS RELATED TO SOIL TEMPERATURE AND TEXTURE AND SIZE OF GRAIN

	Summer per cent	Autumn per cent	Averages per cent	Starchy per cent	Sugary per cent	Larger per cent	Smaller per cent
1. Gold Nugget—Crosby	85.30	56.30	70.82	89.50	52.50	70.35	71.39
2. Voorhees Red—Eight-rowed Pop....	95.80	73.70	84.75	94.00	75.05	87.10	82.40
3. Voorhees Stowell—Golden Queen....	89.00	41.30	65.15	81.60	48.70	61.90	89.00
Averages	90.03	57.10	73.57	88.50	58.60	73.11	80.90

The line of averages shows that the starchy is far ahead of the sugary grain in viability, and the larger grains are less viable than the smaller grains.

Table III gives the differences in other respects.

TABLE III

MESOCOTYL LENGTH, AS RELATED TO SOIL TEMPERATURE AND TEXTURE AND SIZE OF GRAIN

	Summer mm.	Autumn mm.	Averages mm.	Starchy mm.	Sugary mm.	Larger mm.	Smaller mm.
1. Gold Nugget—Crosby	30.65	27.26	28.95	28.62	29.29	29.11	28.79
2. Voorhees Red—Eight-rowed Pop....	34.15	31.11	32.63	33.27	32.00	33.26	31.99
3. Voorhees Stowell—Golden Queen....	33.47	31.87	32.67	32.70	32.65	31.87	33.47
Averages	32.85	30.08	31.41	31.53	31.30	31.41	31.41

The mesocotyl (that is, the first internode formed in the corn seedling, the stem portion that separates the grain from the first joint, where a set of roots soon forms) is remarkably uniform in the three subjects, being practically the same for the two pop corns, and somewhat less for the other. There is somewhat less development in the autumn set, and here it is possible that the full length had not been attained when the harvest was made.

No differences are found associated with the texture or size of the seed.

The mesocotyl is a structure that varies with the amount of light and moisture, and may elongate greatly when the seedling is in a moist, dark place, as when deeply planted, and serves to bring the first node near to the surface, where secondary or anchor roots are developed. It is not a suitable subject for the study of variability.

In Table IV there is shown, of course, a striking difference of 10 days between the average emergence of the summer and autumn series, but when the two results are averaged, the range among the three crosses is but slight, there being a half-day only between the two extremes. In all instances the starchy are quicker in "coming up" than the sugary, the difference being 14 hours. In like measure the smaller seeds are quicker than the larger, but the difference is only 4 hours.

TABLE IV

EMERGENCE, IN DAYS, AS RELATED TO SOIL TEMPERATURE AND TEXTURE AND SIZE OF GRAIN

	Summer days	Autumn days	Averages days	Starchy days	Sugary days	Larger days	Smaller days
1. Gold Nugget—Crosby	4.46	14.27	9.37	9.74	9.74	9.64	9.10
2. Voorhees Red—Eight-rowed Pop....	3.89	14.33	9.11	8.80	9.42	9.25	8.96
3. Voorhees Stowell—Golden Queen....	3.84	15.30	9.57	9.01	10.13	9.42	9.72
Averages	4.06	14.63	9.35	9.18	9.76	9.43	9.26

Under the same conditions in a special test the starchy smaller grains gave an emergence record of 3.81 days, while the sugary larger kernels required 5.04 days, a difference of 25 per cent in time.

TABLE V

LENGTH OF PLANT, AS RELATED TO SOIL TEMPERATURE AND TEXTURE AND SIZE OF GRAIN

	Summer mm.	Autumn mm.	Averages mm.	Starchy mm.	Sugary mm.	Larger mm.	Smaller mm.
1. Gold Nugget—Crosby	44.5	12.6	28.6	30.0	27.0	28.4	28.7
2. Voorhees Red—Eight-rowed Pop....	41.0	11.9	26.5	27.7	25.2	26.4	26.4
3. Voorhees Stowell—Golden Queen....	39.3	10.8	25.1	26.1	23.9	26.2	23.8
Averages	41.6	11.7	26.7	27.9	25.3	27.0	26.3

The summer-grown plants are nearly four times the length of those grown in the autumn as shown in Table V. The variety with the largest grains grew the largest plants, and the starchy out-grew the sugary, possibly because the latter are slow in starting. There was but little difference in length of plants between those from the larger and smaller grains. Here again it is recalled that the small grains start more quickly and the results at the end of this test may not represent the differences that might obtain a month or more later.

In Table VI the great difference is associated with temperature, the weight being 3.4 times as much for the summer as for the autumn series.

The general averages were the same for the pop corn crosses, and were much exceeded by the large-grained cross Gold Nugget-Crosby. The starchy and larger seeds both gave larger plants than the sugary and small-seeded groups.

TABLE VI

WEIGHT OF SEEDLINGS, AS RELATED TO SOIL TEMPERATURE AND TEXTURE AND SIZE OF GRAIN

	Summer gm.	Autumn gm.	Averages gm.	Starchy gm.	Sugary gm.	Larger gm.	Smaller gm.
1. Gold Nugget—Crosby	4.96	1.54	3.25	3.67	2.85	3.34	3.16
2. Voorhees Red—Eight-rowed Pop....	3.83	1.22	2.53	2.71	2.35	2.58	2.47
3. Voorhees Stowell—Golden Queen....	4.04	1.04	2.54	2.87	2.22	2.74	2.35
Averages	4.27	1.27	2.77	3.08	2.47	2.88	2.66

TABLE VII

VIGOR OF SEEDLING, AS RELATED TO SOIL TEMPERATURE AND TEXTURE AND SIZE OF GRAIN

	Summer gm.	Autumn gm.	Averages gm.	Starchy gm.	Sugary gm.	Larger gm.	Smaller gm.
1. Gold Nugget—Crosby	4.64	1.21	2.93	3.29	2.57	2.97	2.88
2. Voorhees Red—Eight-rowed Pop....	3.61	1.01	2.32	2.48	2.16	2.34	2.28
3. Voorhees Stowell—Golden Queen....	3.82	0.83	2.33	2.63	2.03	2.50	2.15
Averages	4.02	1.02	2.53	2.80	2.25	2.60	2.44

TABLE VIII

VARIABILITY IN LENGTH OF SEEDLINGS, AS RELATED TO SOIL TEMPERATURE AND SIZE OF GRAIN

	Summer per cent	Autumn per cent	Averages per cent	Starchy per cent	Sugary per cent	Larger per cent	Smaller per cent
1. Gold Nugget—Crosby	1.11	1.85	1.48	1.28	1.69	1.69	1.29
2. Voorhees Red—Eight-rowed Pop....	1.04	2.00	1.52	1.32	1.73	1.41	1.63
3. Voorhees Stowell—Golden Queen....	1.09	3.10	2.10	1.92	2.28	2.13	2.07
Averages	1.08	2.32	1.70	1.50	1.90	1.73	1.66

As given in Table VII the vigor is expressed in terms of live substance, that is, the weight of the crops less that of the seeds planted. Soil temperature was the chief modifying factor. The results seem to be quite parallel with those for weights of seedlings. The Gold Nugget-Crosby, with its large grains, showed much the greatest growth, the other two crosses being nearly alike. Starchy grains uniformly lead the sugary ones, and the larger ranked above the smaller grains, but less strikingly.

Table VIII indicates that, first of all, variability is somewhat inborn, as may be seen by making a comparison of the second and third cross, the former being less variable than the latter in each of the six tests. From the average it is found that the autumn-grown crop is over twice as variable as the summer one, which had the more favorable temperature and made the larger development. In like manner, the plants from the sugary are more variable than those from the starchy grains.

As an extreme instance, not shown in the tables, the average variability in length for the summer-grown plants from starchy large grains is only 0.89 per cent, while the sugary smaller grains grown in autumn gave a range of 2.85 per cent, showing, in other words, three times as much variability.

Summary

It goes without writing, that the environmental factor of soil temperature is a controlling one in the growth of seedling corn.

Starchy grains of the same ears are much larger (27 per cent) than sugary grains, and more viable (51 per cent), and emerge from a depth of 2 inches nearly a day sooner, showing 25 per cent more vigor and 26 per cent less variability.

The larger grains of the same ears weigh 29 per cent more than the selected smaller, and are only 4 per cent more viable, emerge more slowly by 4 hours, show 7 per cent more vigor and have nearly the same viability as the smaller kernels of the same texture.

One of the features of the present tests is the suggestion, that in somewhat favorable conditions for seedlings there may be a practical method of eliminating the weaker members, thus leaving only those that may yield better final results than when all plants developed from the same lot of seeds, under highly stimulating conditions, are grown. It is a method of selection and an application to crop-growing of the general law of the survival of the fittest—an early sifting out of weaker individuals.

Any conditions of a seed-bed, that tend to bring to light the degree of vigor of the seedlings are essential in the vital test. It is possible, that a lack in any of the three leading physical environmental factors, namely heat, moisture or light, may give the results sought.

With small seeds, like those of tomatoes, eggplants, peppers, etc., it may be that a lack of high soil fertility might suffice in this sifting process.

In an exhaustive study of the kind here begun, all the associated inherited characters carried by the different varieties of seeds need to be fully considered.

STATEMENT

OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC., REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912,

of Soil Science, published monthly at New Brunswick, N. J., for April 1, 1917.

State of New Jersey,

County of Middlesex,

Before me, a Notary Public in and for the State and county aforesaid, personally appeared Jacob G. Lipman, who, having been duly sworn according to law, deposes and says that he is the publisher of SOIL SCIENCE and that the following is, to the best of his knowledge and belief, a true statement of the ownership, management, etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, embodied in section 443, Postal Laws and Regulations, to wit:

1. That the names and addresses of the publisher, editor, managing editor, and business managers are:

Publisher, Jacob G. Lipman, New Brunswick, N. J.

Editor, Jacob G. Lipman, New Brunswick, N. J.

Managing Editor, Carl R. Woodward, New Brunswick, N. J.

Business Manager, Carl R. Woodward, New Brunswick, N. J.

2. That the owners are:

Trustees of Rutgers College, New Brunswick, N. J.

(Signature) JACOB G. LIPMAN.

Sworn to and subscribed before me this 4th day of April, 1917.

IRVING E. QUACKENBOSS,
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